

STN SEARCH

#10/537,746

2/1/2008

=> index bioscience medicine

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE,
AQUASCI, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CAPLUS,
CEABA-VTB, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, DRUGB,
DRUGMONOG2, DRUGU, EMBAL, EMBASE, ...' ENTERED AT 11:26:35 ON 04 FEB 2008

72 FILES IN THE FILE LIST IN STNINDEX

Enter SET DETAIL ON to see search term postings or to view
search error messages that display as 0* with SET DETAIL OFF.

=> S (arabinogalactanase or galactanase or (arabinogalactan (w) endo-1,4-beta-galactosidase))

68 FILE AGRICOLA
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43 FILES HAVE ONE OR MORE ANSWERS, 72 FILES SEARCHED IN STNINDEX

L1 QUE (ARABINOGALACTANASE OR GALACTANASE OR (ARABINOGALACTAN (W) ENDO-1,4-BETA-GALACTOSIDASE))

=> d rank

F1 392 DGENE

F2	368	CAPLUS
F3	287	USPATFULL
F4	248	USGENE
F5	200	BIOSIS
F6	145	SCISEARCH
F7	134	GENBANK
F8	105	BIOTECHABS
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F10	101	PASCAL
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F18	59	FSTA
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F20	52	USPAT2
F21	51	EMBASE
F22	44	BIOTECHNO
F23	39	BIOENG
F24	35	FROSTI
F25	26	TOXCENTER
F26	17	CEABA-VTB
F27	6	DISSABS
F28	4	AQUASCI
F29	2	CIN
F30	2	CONFSCI
F31	2	CROPB
F32	2	CROPU
F33	2	DRUGU
F34	2	NTIS
F35	2	OCEAN
F36	2	VETU
F37	2	WPIFV
F38	1	ANTE
F39	1	DDFB
F40	1	DDFU
F41	1	DRUGB
F42	1	RDISCLOSURE
F43	1	NLDB

=> file f2, f3, f5-f6, f8-f12

FILE 'CAPLUS' ENTERED AT 11:29:12 ON 04 FEB 2008
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FILE 'BIOTECHABS' ACCESS NOT AUTHORIZED

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FILE 'WPIDS' ENTERED AT 11:29:12 ON 04 FEB 2008
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=> S L1

L2 1396 L1

=> S (gene or sequence or polynucleotide or clone or recombinant) (s) L2

L3 220 (GENE OR SEQUENCE OR POLYNUCLEOTIDE OR CLONE OR RECOMBINANT)
(S) L2

=> S (mutant or variant) (s) L3

L4 38 (MUTANT OR VARIANT) (S) L3

=> dup rem L4

PROCESSING COMPLETED FOR L4

L5 31 DUP REM L4 (7 DUPLICATES REMOVED)

=> d ibib abs L5 1-31

L5 ANSWER 1 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

DUPLICATE 1

ACCESSION NUMBER: 2007-10690 BIOTECHDS <<LOGINID::20080204>>

TITLE: Novel isolated mature functional polypeptide obtainable from
Bacillus species P203, useful for industrial or household
technical process, for cleaning cellulosic fabric, preparing
food or feed additive;
involving plasmid vector-mediated gene expression in
Escherichia coli, useful for development of a surfactant
and for a biocatalysis application

AUTHOR: NIELSEN P; BORCHERT M

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2007019859 22 Feb 2007

APPLICATION INFO: WO 2006-DK447 15 Aug 2006

PRIORITY INFO: DK 2005-1156 16 Aug 2005; DK 2005-1156 16 Aug 2005

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2007-301752 [29]

AN 2007-10690 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - An isolated mature functional polypeptide obtainable from
bacterium strain Bacillus sp. P203 deposited under accession number DSM
17419, and encoded by polynucleotides comprised in the genome of strains
of Bacillus sp. such as Bacillus plankticiensis deposited under accession
number DSM 17419, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the
following: (1) a ***polynucleotide*** having nucleotide

sequence encoding the polypeptide; (2) a composition comprising
the polypeptide or the ***polynucleotide***; (3) preparing the
composition, involves admixing the polypeptide with an excipient; (4) a
nucleic acid construct comprising the nucleotide ***sequence***
operably linked to one or more control sequences that direct the
production of the polypeptide in a host cell; (5) a ***recombinant***
expression vector comprising the nucleic acid construct; (6) a

recombinant host cell comprising the nucleic acid construct; (7)
producing the polypeptide; (8) a storage medium suitable for use in an
electronic device, comprising information of the amino acid

sequence of the polypeptide or the nucleotide ***sequence***
of the ***polynucleotide***; and (9) a process involves employing the
polypeptide or the ***polynucleotide*** in an industrial or household
technical process.

BIOTECHNOLOGY - Preparation (claimed): Preparation of the
polypeptide, involves (a) cultivating a strain, which in its wild-type
form is capable of producing the polypeptide, to produce the polypeptide,
and recovering the polypeptide, or (b) cultivating the

recombinant host cell under conditions conducive for production
of the polypeptide, and recovering the polypeptide. The method involves
fusing genes from the genome of the strain Bacillus sp. P203 deposited

under accession number DSM 17419 with a ***gene*** encoding a signalless reporter, through transposon tag, growing host cell clones having fused ***gene*** of the strain Bacillus sp. P203 (DSM 17419) in a medium revealing the presence of the reporter, detecting clones secreting the reporter, and isolating ***gene*** and polypeptide of the strain Bacillus sp. P203 (DSM 17419) comprised in that ***clone*** Preferred Polypeptide: The polypeptide is chosen from polypeptide comprising an amino acid ***sequence*** having at least 70% identity with a ***sequence*** of a mature polypeptide having a fully defined 651, 276, 234, 360, 504 and 534 amino acids sequences (SEQ ID No. 2-12 (even SEQ ID numbers)) ***sequence*** given in the specification, or its fragment, and a polypeptide encoded by a nucleotide ***sequence*** capable of hybridizing under high stringency conditions with a ***polynucleotide*** probe chosen from the complementary strand to a nucleotide ***sequence*** chosen from regions of a fully defined 1953, 828, 702, 1080, 2712 and 2804 nucleotide sequences (SEQ ID No. 1-11 (odd SEQ ID numbers)) ***sequence*** given in the specification encoding a mature polypeptide and the complementary strand to the cDNA ***sequence*** contained in a nucleotide sequences chosen from SEQ ID No. 1-11 (odd SEQ ID numbers) encoding a mature polypeptide, where the polypeptide has function of the corresponding mature polypeptides comprised in SEQ ID No. 2-12 (even SEQ ID numbers). The polypeptide is an enzyme having function chosen from xylanase, serine protease, carbonic anhydrase, rhamnogalacturonan lyase and ***galactanase***. The enzyme is chosen from an enzyme comprising amino acid ***sequence*** having at least 70% identity with an amino acid ***sequence*** chosen from mature enzymes comprised in SEQ ID No. 2-10 (even SEQ ID numbers) or its fragment, and an enzyme, which is encoded by a nucleotide ***sequence*** capable of hybridizing under high stringency conditions with a ***polynucleotide*** probe chosen from the complementary strand to a nucleotide ***sequence*** chosen from regions of SEQ ID No. 1-11 (odd SEQ ID numbers) encoding the mature enzyme and complementary strand to the cDNA ***sequence*** contained in a nucleotide sequences chosen from regions of SEQ ID No. 1-11 (odd SEQ ID numbers) encoding the mature polypeptide, where the enzyme has function of the corresponding mature polypeptides comprised in SEQ ID No. 2-12 (even SEQ ID numbers). The ***polynucleotide*** encoding the polypeptide, consists of a nucleotide ***sequence*** chosen from regions of SEQ ID No. 1-11 (odd SEQ ID numbers) encoding a mature polypeptide or a ***sequence*** differing from it by virtue of the degeneracy of the genetic code. The isolated enzyme is chosen from (a) an enzyme comprising an amino acid ***sequence*** having at least 70% identity with amino acid ***sequence*** of mature enzyme chosen from xylanase, serine protease, carbonic anhydrase, rhamnogalacturonan lyase and ***galactanase*** secreted from the strain Bacillus sp. P203 deposited under DSM 17419, or its fragment, and (b) a polypeptide encoded by nucleotide ***sequence*** capable of hybridizing under high stringency conditions with ***polynucleotide*** probe chosen from the complementary strand to nucleotide ***sequence*** comprised in the strain of Bacillus sp. P203 deposited under accession number DSM 17419 encoding a mature enzyme chosen from xylanase, serine protease, carbonic anhydrase, rhamnogalacturonan lyase and ***galactanase*** secreted from that strain and the complementary strand to the cDNA ***sequence*** contained in a nucleotide sequences comprised in the strain of Bacillus sp. P203 deposited under accession number DSM 17419 encoding mature enzyme chosen from xylanase, serine protease, carbonic anhydrase, rhamnogalacturonan lyase and ***galactanase*** secreted from that strain, where the enzyme has a function chosen from xylanase, serine protease, carbonic anhydrase, rhamnogalacturonan lyase and ***galactanase***. Preferred Composition: The composition comprises at least two different polypeptides, more preferably at least 15, and most preferably at least 20 different polypeptides. The composition comprises all polypeptides secreted when fermenting a sample of strain Bacillus sp. P203 DSM 17419 or its ***mutant***, where one or more genes has been deleted or added. The composition further comprises one or more additional enzymes. The composition characterized by being a detergent composition which, in addition to the polypeptide, comprises surfactant. The composition characterized by being a feed composition which in addition to the polypeptide comprises a cereal or grain product. The composition characterized by being a food composition. The composition

further comprises polysaccharide or its mixture.

USE - For industrial or household technical process (claimed), for cleaning a cellulosic fabric, preparing food or feed additive, treatment of lignolitic materials and pulp, promoting water purification or mineralization from aqueous media and for carbon fixation from carbon dioxide emission streams, and as target for drug development such as sulphonamide derivatives.

EXAMPLE - Chromosomal DNA from strain Bacillus sp. P203 (DSM 17419) was prepared. The prepared DNA was partially cleaved with MboI and separated in a sucrose gradient by ultracentrifugation. Fragments of 3-10 kilobases were extracted, precipitated and resuspended in a suitable buffer. The resulting Escherichia coli colonies were pooled and plasmids were prepared. The eluate of approximately 1 ml containing the plasmid DNA was precipitated in a centrifuge with sodium acetate and 96% ethanol at 20000 rpm at 4 degrees C, washed with 70% ethanol, dried at room temperature and resuspended in TE buffer. The genes obtained by the transposon assisted signal trapping (TAST) encoded secreted functional polypeptides. An electrocompetent E.coli DH10B cells were transformed by electroporation (transposon tagged plasmid pool (5 μl) was mixed with SOC medium (1 ml), pre-incubated for 1 hour at 37 degrees C and plated on LB with ampicillin, kanamycin and chloramphenicol and incubated for 2-3 days at 30 degrees C). Templates for DNA sequencing from the signal-trapped library were prepared by rolling circle amplification. The transposon tagged plasmids were sequenced with primer A2up agcggttgccgcgcgatcc (SEQ ID No. 13) which read upstream into the transposon tagged ***gene***, and with B primer ttaattcggtcgaaaaggatcc (SEQ ID No. 14), which read downstream into the transposon, tagged ***gene***. Thus, the obtained sequences were functional genes, which encoded intact and functional polypeptides.(85 pages)

L5 ANSWER 2 OF 31 USPATFULL on STN

ACCESSION NUMBER: 2007147087 USPATFULL <<LOGINID::20080204>>

TITLE: Galactanase variants

INVENTOR(S): De Maria, Leonardo, Frederiksberg, DENMARK

PATENT ASSIGNEE(S): Novozymes A/S, Bagsvaerd, DENMARK, DK-2880 (non-U.S. corporation)

NUMBER KIND DATE

PATENT INFORMATION: US 2007128180 A1 20070607

APPLICATION INFO.: US 2003-537746 A1 20031211 (10)
WO 2003-DK851 20031211
20050606 PCT 371 date

NUMBER DATE

PRIORITY INFORMATION: DK 2002-1968 20021220
DK 2003-537 20030408

DOCUMENT TYPE: Utility

FILE SEGMENT: APPLICATION

LEGAL REPRESENTATIVE: NOVOZYMES NORTH AMERICA, INC., 500 FIFTH AVENUE, SUITE 1600, NEW YORK, NY, 10110, US

NUMBER OF CLAIMS: 19

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 174 Drawing Page(s)

LINE COUNT: 1890

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Variants of Glycoside Hydrolase family 53 galactanases, e.g. variants of the galactanases from strains of Yersinia, Aspergillus, Humicola, Meripilus, Myceliophthora, Thermomyces, Bacillus, Bifidobacterium, Cellvibrio, Clostridium, Pseudomonas, Thermotoga, or Xanthomonas.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 3 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2007-22503 BIOTECHDS <<LOGINID::20080204>>

TITLE: New thermophilic Talaromyces emersonii strain which actively secretes enzymes, useful in waste reduction, for producing food ingredients and biofuels, in textile processing and recycling, or in antifungal, biocontrol and slime control;

thermophilic fungus enzyme for use in food industry and
natural antioxidant

AUTHOR: TUOHY M G; MURRAY P G; GILLERAN C T; COLLINS C M; REEN F J;
MCLOUGHLIN L P; LYDON A G S; MALONEY A P; HENEIGHAN M N;
ODONOGHUE A J; MAHON C S

PATENT ASSIGNEE: UNIV NAT IRELAND GALWAY

PATENT INFO: WO 2007091231 16 Aug 2007

APPLICATION INFO: WO 2007-IE16 9 Feb 2007

PRIORITY INFO: IE 2006-90 10 Feb 2006; IE 2006-90 10 Feb 2006

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2007-690271 [64]

AN 2007-22503 BIOTECHDHS <<LOGINID::20080204>>

AB DERVENT ABSTRACT:

NOVELTY - A thermophilic strain of *Talaromyces emersonii*, which has a growth temperature of 30-90degreesC and which actively secretes enzymes at temperatures above 55degreesC, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are: (1) an enzyme produced by a strain above which retains activity at temperatures above 55degreesC; (2) an enzyme composition comprising a cellobiohydrolase I or a cellobiohydrolase II or their mixture, beta-glucosidase I, xylanase and endo-beta-(1,3)4-glucanase; (3) bio-ethanol or bio-gas whenever produced by a process of the invention; and (4) a method for obtaining an enzyme system for converting a target substrate.

BIOTECHNOLOGY - Preferred Strain: The strain of *T. emersonii* has the deposition no. NJ 393751 or its ***mutant*** also encoding

thermostable enzymes. Preferred Enzyme: The enzyme is carbohydrate-modifying enzymes, proteolytic enzymes, oxidases, or oxidoreductases. A xylanase has a molecular weight of 17.5 kDa, pH optimum of 4-4.5, retaining 91% activity at pH 3.0, and has degrading activity against both mixed-link D-xylans and mixed-link D-glucans. The xylanase also has activity against aryl-beta-xylosides. The xylanase is derived from the *T. emersonii* strain with the deposition no. IMI 393751, or a strain substantially similar to it or its ***mutant***.

Preferred Enzyme Composition: The composition comprises 0.5-90% cellobiohydrolase I or its cellobiohydrolase flora mixture, 0.1-33% beta-glucosidase I, 0.6-89% xylanase and 0.4-68% endo-beta-(1,3)4-glucanase. An enzyme composition comprises CBH1 (10-30%), CBH11 (10-15%), beta-(1,3)4-glucanase (20-45%), beta-glucosidase (2-15%), and Xylanase (18-55%), where the enzyme composition further comprises one or more of the following: 0.1-20.0% beta-Xylosidase, 0.1-10.0% alpha-Glucuronidase, 1-15% exoxylanase, 0.1-5.0% alpha-L-Arabinofuranosidase, 2-25% pectinolytic enzymes, 5-10% hemicellulases, 5-10% starch modifying enzymes, 1-10% oxidoreductase/oxidase and esterases; and 7-25% protease. An enzyme composition comprises CBH I (1-25%), CBH II (1-28%), beta(1,3)4-glucanase (18-40%), beta-glucosidase (2-30%), Xylanase (15-55%), and beta-xylosidase (0.7-20%), where the enzyme composition further comprises one or more of the following: alpha-Glucuronidase (1-10%), alpha-L-Arabinofuranosidase (0.1-5.0%), 1-15% exoxylanase, 5-25% pectinolytic enzymes, 2-12% starch modifying activity, 2-11% hemicellulases, 1-15% oxidoreductases/oxidase and esterases and 2-15%. An enzyme composition comprises CBH I (12-55%), CBH II (15-30%), beta(1,3)4-glucanase (12-26%), beta-glucosidase (5-12%), Xylanase (5-30%), beta-Xylosidase (0.1-10%) and alpha-L-Arabinofuranosidase (0.5-3.0%), where the enzyme composition further comprises one or more of the following: alpha-Glucuronidase, 5-15%, Other hydrolases including Pectinolytic enzymes Phenolic acid and acetyl(xylan)esterases Protease and Lignin-modifying oxidase activities, proteases and oxidases. An enzyme composition comprises CBH I (15-30%), CBH II (10-40%), beta(1,3)4-glucanase (15-40%), beta-glucosidase (2-15%), Xylanase (15-30%), and 1-8% beta-Xylosidase, where the enzyme composition further comprises one or more of the following: Exoxylanase; beta-Glucuronidase; alpha-L-Arabinofuranosidase; pectinolytic enzymes, including galactosidases, rhamnogalacturonase, polygalacturonase, exogalacturonase and ***galactanase***; starch modifying activity; other hemicellulases, including galactosidases; oxidoreductase oxidase and esterases; and protease. An enzyme composition comprises CBH I (5-55%), CBH II (8-50%), beta(1,3)4-glucanase (10-30%), beta-glucosidase (0.5-30%), Xylanase (5-30%), and beta-Xylosidase (0.1-10%), where the enzyme composition further comprises one or more of the following: alpha-L-Arabinofuranosidase; alpha-glucuronidase; other hydrolases,

including selected Pectinolytic enzymes, esterases; Protease; and oxidases. An enzyme composition comprises CBH I (2-10%), CBH II (2-10%), (1,3)4-glucanase(10-45%), beta-glucosidase (5-10%), and Xylanase (1-30%), where the enzyme composition further comprises one or more of the following: N-Acetylglucosaminidase; chitinase; beta-Xylosidase; alpha-glucuronidase; alpha-L-Arabinofuranosidase; pectinolytic enzymes, including galactosidases, rhamnogalacturonase, polygalacturonase, exogalacturonase and ***galactanase*** ; starch modifying activity; other hemicellulases, including galactosidases; oxidoreductase/oxidase and esterases; and protease. An enzyme composition comprises CBH I (1-20%), CB II (1-28%), beta(1,3)4-glucanase (15-40%), beta-glucosidase (2-15%); Xylanase (18-55%), beta-Xylosidase (0.1-10%) and alpha-L-Arabinofuranosidase (0.5-5.0%), where the enzyme composition further comprises one or more of the following: alpha-Glucuronidase; starch modifying activity; other hemicellulases, including galactosidases; oxidoreductase/oxidase and esterases; protease; exoxylanase; Other hydrolases, including Pectinolytic enzymes, Phenolic acid and acetyl(xylan)esterases; Protease; and Lignin modifying oxidase activities. An enzyme composition comprises CBH I (5-30%), CBH II (1-15%), beta(1,3)4-glucanase (10-40%), beta-glucosidase (2-15%), Xylanase (18-48%), 0.1-20% beta-Xylosidase, 1-10%, alpha-Glucuronidase and 0.1-5.0 % alpha-L-Arabinofuranosidase, where the enzyme composition further comprises one or more of the following: Exoxylanase; pectinolytic enzymes, including galactosidases, rhamnogalacturonase, polygalacturonase, exogalacturonase and ***galactanase*** ; starch modifying activity; other hemicellulases, including galactosidases; oxidoreductase/oxidase and esterases and protease. An enzyme composition comprises CBH I (0.5-10%), CBH II (0.5-10%), beta(1,3)4-glucanase (15-43%), beta-glucosidase (2-10%), Xylanase (30-88%), 0.1-2.0 %-Xylosidase, 0.1-3.0 % alpha-Glucuronidase, 0.1-4.0 % alpha-L-arabinofuranosidase, where the enzyme composition further comprises one or more of the following: pectinolytic enzymes; starch modifying activity; oxidoreductase/oxidase and esterases; and protease, where the enzyme composition further comprises CBH I (3-15%), CBH II (3-15%), (1,3)4-glucanase (25-45%), alpha-glucosidase (2-15%), Xylanase (18-55%), 0.5-7.0% beta-Xylosidase, 0.5-10% alpha-Glucuronidase, and 0.1-5.0 % alpha-L-Arabinofuranosidase, and where the enzyme composition further comprises one or more of the following: Exoxylanase; pectinolytic enzymes, including galactosidases, rhamnogalacturonase, polygalacturonase, exogalacturonase and ***galactanase*** ; starch modifying activity; other hemicellulases, including galactosidases; oxidoreductase/oxidase and esterases; and protease. An enzyme composition comprises CBH I (1-15%), CBH II (1-15%), beta(1,3)4-glucanase (10-45%), alpha-glucosidase (2-10%), Xylanase (1-55%), 0.5-1.2% beta-Xylosidase, where the enzyme composition further comprises one or more of the following: alpha-Glucuronidase, alpha-L-Arabinofuranosidase; chitinase pectinolytic enzymes, including galactosidases, rhamnogalacturonase, polygalacturonase, exogalacturonase and ***galactanase*** ; starch modifying activity; other hemicellulases, including galactosidases; oxidoreductase/oxidase and esterases; protease. An enzyme composition comprises CBH I (1-10%), CBH II (5-15%), beta(1,3)4-glucanase (15-40%), alpha-glucosidase (2-30%), Xylanase (15-55%), 1-12% beta-Xylosidase, 1-8%, alpha-Glucuronidase and 0.5-5.0% alpha-L-Arabinofuranosidase, where the enzyme composition further comprises one or more of the following: pectinolytic enzymes, including galactosidases, rhamnogalacturonase, polygalacturonase, exogalacturonase and ***galactanase*** ; starch modifying activity; other hemicellulases, including galactosidases; oxidoreductase/oxidase and esterases; protease. An enzyme composition comprises CBH I (1-20%), CBH II (1-40%), (1,3)4-glucanase (15-45%), alpha-glucosidase (2-30%), Xylanase (10-55%), 0.5-10% beta-Xylosidase, 0.1-5% alpha-L-Arabinofuranosidase, where the enzyme composition further comprises one or more of the production or biopharmaceuticals bioactive polysaccharides, glycopeptides and flavonoid glycosides from terrestrial and marine plants, plant residues, fungi and waste streams or by-products rich in simple sugars. An enzyme composition comprises CBH I (1-20%), CBH II (1-40%), (1,3)4-glucanase (15-45%), beta-glucosidase (2-12%), Xylanase (1-35%), and beta-Xylosidase (1-5%), where the enzyme composition further comprises one or more of the following: alpha-Glucuronidase (0.5-10%), alpha-L-Arabinofuranosidase (1-5%), 1-10% exoxylanase, 5-25% pectinolytic

enzymes, 2-15% starch modifying activity, 5-10% hemicellulases, 1-15% oxidoreductases/oxidase and esterases and 5-15% protease. An enzyme composition comprises CBH I (3-15%), CBH II (3-15%), beta(1,3)4-glucanase (25-45%), beta-glucosidase (2-15%), Xylanase (10-30%), and beta-Xylosidase (0.5-8%), where the enzyme composition further comprises one or more of the following: alpha-Glucuronidase (0.5-10%), alpha-L-Arabinofuranosidase (0.1-5%), 1-5% exoxylanase, 2-15% pectinolytic enzymes, 2-7% starch modifying activity, 5-10% hemicellulases, 1-10% oxidoreductases/oxidase and esterases and 5-15% protease. An enzyme composition comprises CBH I (1-25%), CBH II (1-40%), beta(1,3)4-glucanase (15-40%), beta-glucosidase (2-15%), Xylanase (18-35%), and beta-Xylosidase (0.5-12%) where the enzyme composition further comprises one or more of the following: alpha-Glucuronidase (0.5-10%), alpha-L-Arabinofuranosidase (0.5-5%), 5-25% pectinolytic enzymes, 2-12% starch modifying activity, 2-10% hemicellulases, 1-15% oxidoreductases/oxidase and esterases and 3-15% protease. An enzyme composition comprises CBH I (1-30%), CBH II (1-40%), beta(1,3)4-glucanase (15-40%), beta-glucosidase (2-15%), Xylanase (18-35%), and beta-Xylosidase (0.5-8%), where the enzyme composition further comprises one or more of the following: alpha-Glucuronidase (0.5-10%), alpha-L-Arabinofuranosidase (1-5%), 5-25% pectinolytic enzymes, 5-12% starch modifying activity, 3-10% hemicellulases, 1-15% oxidoreductases/oxidase and esterases, 1-12% exoxylanase and 5-15% protease. An enzyme composition comprises CBH I (0.4-15%), CBH II (0.4-15%), beta(1,3)4-glucanase (7-45%), beta-glucosidase (2-25%), Xylanase (1-78%), and beta-Xylosidase (0.4-8%), where the enzyme composition further comprises one or more of the following: alpha-Glucuronidase (0.5-4%), alpha-L-Arabinofuranosidase (0.1-5%), 2-25% pectinolytic enzymes, 2-15% starch modifying activity, 2-10% hemicellulases, 1-15% oxidoreductases/oxidase and esterases, 2-12% exoxylanase and 5-30% protease. An enzyme composition comprises CBH I (0.4-30%), CBH II (0.4-10%), (1,3)4-glucanase (7-45%), beta-glucosidase (2-12%), Xylanase (1-78%), and beta-Xylosidase (0.1-8%), where the enzyme composition further comprises one or more of the following: alpha-Glucuronidase (0.1-2%), alpha-L-Arabinofuranosidase (0.1-5%), 2-10% pectinolytic enzymes, 2-15% starch modifying activity, 2-10% hemicellulases, 1-10% oxidoreductases/oxidase and esterases, 2-5% exoxylanase and 8-15% protease. An enzyme composition comprises CBH I (0.4-15%), CBH II (0.4-30%), beta(1,3)4-glucanase (7-45%), beta-glucosidase (P12%), Xylanase (10-78%), and beta-Xylosidase (0.4-8%), where the enzyme composition further comprises one or more of the following: alpha-Glucuronidase (0.5-10%), alpha-L-Arabinofuranosidase (0.1-5%), 2-15% pectinolytic enzymes, 2-8% starch modifying activity, 5-10% hemicellulases, 1-10% oxidoreductases/oxidase and esterases, and 5-15% protease. Preferred Method: Obtaining an enzyme system for converting a target substrate comprises: (A) obtaining a sample of the target substrate; (B) allowing an inoculum of a microorganism strain to grow on the target substrate and secrete enzymes; (C) recovering the enzymes secreted during growth on the target substrate; (D) determining enzyme activities and enzyme properties; (E) constructing a ***gene*** expression profile; (F) identifying enzyme proteins and constructing a protein expression profile; (G) comparing the ***gene*** expression with the protein expression profile; and (H) purifying the enzymes. The method further comprises analyzing the enzymes, and designing the enzyme system.

USE - The enzyme, enzyme composition, or microorganism strain above is useful: for bioconversion of plant or plant-derived materials or waste streams including hospital waste; for producing monosaccharide-rich feedstocks from plant residues; in processing and recycling of wood, paper products and paper, in textile processing and recycling; in the saccharification of paper wastes; in antifungal, biocontrol and slime control; for horticultural applications; in animal feed production to enhance the digestibility of cereal-based feedstuffs; in producing low pentose-containing cereal-based feedstuffs for monogastric animals with improved digestibility and low non-cellulosic beta-glucans; in producing functional feedstuffs with bioactive potential for use in veterinary healthcare; in producing specialized dairy or dietary products, e.g. foodstuffs and beverage formulations for geriatric and infant healthcare; in the bakery and confectionary sectors, and in the formulation of novel health food bakery products; to increase the bioavailability of

biomolecules with natural anti-bacterial and anti-viral activity, including flavonoid and cyanogenic glycosides, saponins, oligosaccharides and phenolics (including ferulic, and p-coumaric acids, epicatechin, catechin, pyrogalllic acid and the like); to increase the bioavailability of natural antioxidant biomolecules, e.g. carotenoids, lycopenes, xanthophylls, anthocyanins, phenolics and glycosides from all plants materials, residues, wastes, including various fruits and berries; in generating feedstocks from raw plant material, and wastes for use in microbial production of antibiotics by fungi and bacteria, including Penicillium sp. and Streptomyces sp.; for generating feedstocks from raw plant materials, plant residues and wastes for use in microbial production of citric acid; for producing oligosaccharides from algal polysaccharides (e.g. laminaran and fucoidan) and additives derived from plant extracts, by generally regarded as safe processes, in the formulation of cosmetics; for producing oligosaccharides and glycopeptides for use as research reagents, in biosensor production and as tools in functional glycomics to probe, receptor-ligand interactions and in the production of substrate libraries to profile enzyme; for producing modified cellulose and beta-glucans, celooligosaccharides, modified starches and maltooligosaccharides, lactulose and polyols (e.g. mannitol, glucitol or dulcitol, xylitol, arabinol); as a feedstock in the production of biofuel; or for altering the calorific value of a waste stream (all claimed).

ADVANTAGE - The present invention provides enzyme preparations which work at higher reaction temperatures, which allows shorter reaction times/enzymatic treatment steps allows simultaneous pasteurization of the hydrolysate, results in significant overall hydrolysis/saccharification, has a potential for reducing enzyme loading and/or a potential for recycling of the enzyme preparation, thus reduce costs associated with the use of these enzymes.

EXAMPLE - No suitable example given.(149 pages)

L5 ANSWER 4 OF 31 USPATFULL on STN

ACCESSION NUMBER: 2006101389 USPATFULL <<LOGINID::20080204>>

TITLE: Whole cell engineering by mutagenizing a substantial portion of a starting genome, combining mutations, and optionally repeating

INVENTOR(S): Short, Jay M., Rancho Santa Fe, CA, UNITED STATES

PATENT ASSIGNEE(S): Diversa Corporation, San Diego, CA, UNITED STATES (U.S. corporation)

NUMBER KIND DATE

PATENT INFORMATION: US 7033781 B1 20060425

APPLICATION INFO.: US 2000-677584 20000930 (9)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2000-594459, filed on 14 Jun 2000, PENDING Continuation-in-part of Ser. No. US 2000-552289, filed on 9 Mar 2000, Pat. No. US 6358709 Continuation-in-part of Ser. No. US 2000-498557, filed on 4 Feb 2000, PENDING Continuation-in-part of Ser. No. US 2000-495052, filed on 31 Jan 2000, PENDING

NUMBER DATE

PRIORITY INFORMATION: US 1999-156815P 19990929 (60)

DOCUMENT TYPE: Utility

FILE SEGMENT: GRANTED

PRIMARY EXAMINER: Park, Hankyel T.

LEGAL REPRESENTATIVE: Love, Jane M., Hale and Dorr LLP

NUMBER OF CLAIMS: 24

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 30 Drawing Figure(s); 28 Drawing Page(s)

LINE COUNT: 36686

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An invention comprising cellular transformation, directed evolution, and screening methods for creating novel transgenic organisms having desirable properties. Thus in one aspect, this invention relates to a method of generating a transgenic organism, such as a microbe or a plant, having a plurality of traits that are differentially activatable.

Also, a method of retooling genes and gene pathways by the introduction of regulatory sequences, such as promoters, that are operable in an intended host, thus conferring operability to a novel gene pathway when it is introduced into an intended host. For example a novel man-made gene pathway, generated based on microbially-derived progenitor templates, that is operable in a plant cell. Furthermore, a method of generating novel host organisms having increased expression of desirable traits, recombinant genes, and gene products.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 5 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2006-16294 BIOTECHDS <<LOGINID::20080204>>

TITLE: New polypeptide having phosphatase activity, useful
(preferably together with phytase) for degrading phytate
and/or inositol phosphates, particularly in animal feeds;
recombinant enzyme protein production via plasmid
expression in host cell for use in phytate degradation and
animal feed

AUTHOR: HATZACK F; GLITSO V; FRISNER H; SASA M; LASSEN S F

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2006063588 22 Jun 2006

APPLICATION INFO: WO 2005-DK787 13 Dec 2005

PRIORITY INFO: DK 2004-1921 13 Dec 2004; DK 2004-1921 13 Dec 2004

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2006-433471 [44]

AN 2006-16294 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide having phosphatase activity is new.

DETAILED DESCRIPTION - An isolated polypeptide having phosphatase activity is selected from: (i) a polypeptide having an amino acid ***sequence*** which has at least 65% identity with amino acids 8-523 of a ***sequence*** comprising 540 amino acids (SEQ ID NO: 4); (ii) a polypeptide which is encoded by a ***polynucleotide*** which has at least 65% identity with nucleotides 73-1620 of a ***sequence*** comprising 1620 bp (SEQ ID NO: 3); (iii) a polypeptide which is encoded by a ***polynucleotide*** which hybridizes under at least medium-high stringency conditions with (a) nucleotides 73-1620 of SEQ ID NO: 3, or (b) a complementary strand of (a); (iv) a ***variant*** comprising a conservative substitution, deletion, and/or insertion of no more than fifty amino acids of amino acids 1-523 of SEQ ID NO: 4; or (v) a fragment of amino acids 1-523 of SEQ ID NO: 4 which contains at least 400 amino acids residues. INDEPENDENT CLAIMS are also included for: (1) an isolated ***polynucleotide*** comprising a nucleotide ***sequence*** which encodes the polypeptide above, the ***polynucleotide*** is selected from: (i) a ***polynucleotide*** encoding a polypeptide having an amino acid ***sequence*** which has at least 65% identity with amino acids 8-523 of SEQ ID NO: 4, (ii) a ***polynucleotide*** having at least 65% identity with nucleotides 73-1620 of SEQ ID NO: 3, (iii) a ***polynucleotide*** which hybridizes under at least medium-high stringency conditions with nucleotides 73-1620 of SEQ ID NO: 3, or (iv) a subsequence of nucleotides 52-1620 of SEQ ID NO: 3 which contains at least 1200 nucleotides; (2) a nucleic acid construct comprising the ***polynucleotide*** operably linked to one or more control sequences that direct the production of the polypeptide in an expression host; (3) a ***recombinant*** expression vector comprising the nucleic acid construct of (2); (4) a ***recombinant*** host cell comprising the nucleic acid construct of (2); (5) a method for producing the polypeptide above; (6) a transgenic plant, plant part or plant cell, which has been transformed with a ***polynucleotide*** encoding the polypeptide above; (7) a transgenic, non-human animal, or their products, or elements, capable of expressing a polypeptide above; (8) improving the nutritional value of an animal feed, where at least one polypeptide above is added to the feed, preferably together with a phytase; (9) an animal feed additive comprising at least one polypeptide above, and at least one fat soluble vitamin, at least one water soluble vitamin, and/or at least one trace mineral; (10) an animal feed composition having a crude protein content of 50-800 g/kg and comprising at least one polypeptide above, and further, preferably, a phytase; and (11) a method for treating vegetable

proteins.

BIOTECHNOLOGY - Preferred Method: Producing the phosphatase polypeptide comprises cultivating a wild-type cell that naturally expresses the enzyme, or a ***recombinant*** cell engineered to do so, and recovering the polypeptide. Treating vegetable proteins comprises adding at least one polypeptide above, preferably together with a phytase, to at least one vegetable protein or protein source. Soybean is included amongst the at least one vegetable protein source. Preferred Animal Feed Additive: The animal feed additive further comprises amylase, acid phosphatase, phytase, xylanase, ***galactanase***, alpha-galactosidase, protease, phospholipase, and/or beta-glucanase.

USE - The polypeptide, preferably together with a phytase, is used in animal feed, and/or useful for degrading phytate and/or inositol phosphates in vivo or in vitro (claimed).

ADVANTAGE - Phytase may be added to animal feeds to degrade phytate to release the phosphate it contains, and metal ions and proteins that are bound to it. The treatment reduces the need to supplement food with phosphorus and other minerals. The use of the new phosphatase in combination with phytase increases the extent of degradation of phytate and inositol phosphates.

EXAMPLE - No relevant example given.(86 pages)

L5 ANSWER 6 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2006-13159 BIOTECHDS <<LOGINID::20080204>>

TITLE: Novel isolated polypeptide having phytase activity, useful in animal feed, in preparation of composition for use in animal feed, and for improving nutritional value of animal feed; the production of a recombinant phytase useful as a feed-additive

AUTHÖR: TAKAMIYA M; SJOHOLM C; FRISNER H; NORGAARD A; SORENSEN M B

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2006037328 13 Apr 2006

APPLICATION INFO: WO 2005-DK632 4 Oct 2005

PRIORITY INFO: DK 2004-1513 4 Oct 2004; DK 2004-1513 4 Oct 2004

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2006-316323 [33]

AN 2006-13159 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide (I) having phytase activity, chosen from polypeptide (P1) comprising an amino acid ***sequence*** having at least 98.6% identity with amino acids 1-411 of fully defined 433 amino acid (SEQ ID No. 2) ***sequence*** given in specification and/or mature polypeptide portion of SEQ ID No. 2, a ***variant*** of P1 comprising a deletion, insertion and/or conservative substitution of one or more amino acids, and a fragment of P1, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for: (1) an isolated ***polynucleotide*** (II) comprising a nucleotide

sequence encoding (I) having phytase activity, chosen from a ***polynucleotide*** encoding P1, and a ***polynucleotide*** having at least 98.3% identity with nucleotides 67-1299 of a fully defined 1302 nucleotide (SEQ ID No. 1) ***sequence*** given in the specification; (2) a nucleic acid construct (III) comprising (II) operably linked to one or more control sequences that direct the production of the polypeptide in an expression host; (3) a ***recombinant*** expression vector comprising (III); (4) a ***recombinant*** host cell comprising (III); (5) producing (I); (6) a transgenic plant, plant portion or plant cell, which has been transformed with (II); (7) a transgenic, non-human animal, or products, or elements thereof, being capable of expressing (I); (8) an animal feed additive (IV) comprising one or more of (I), one or more of fat soluble vitamin, one or more of water soluble vitamin, and/or one or more of trace mineral; and (9) an animal feed composition having a crude protein content of 50-800 g/kg and comprising one or more of (I).

BIOTECHNOLOGY - Preparation: (I) is produced by: (a) cultivating a cell, which in its wild-type form is capable of producing (I), under conditions conducive for production of (I), and recovering (I); or (b) cultivating a ***recombinant*** host cell comprising (III) having a nucleotide ***sequence*** encoding (I) under conditions conducive for production of (I), and recovering (I) (claimed). Preferred Polypeptide: (I) is chosen from a polypeptide comprising an amino acid

sequence which has at least 99.1% identity with amino acids 1-411 of a fully defined 433 amino acid (SEQ ID No. 4) ***sequence*** given in the specification and/or the mature polypeptide portion of SEQ ID No. 4, a ***variant*** of the above polypeptide comprising a deletion, insertion, and/or conservative substitution of one or more amino acids, and a fragment of the above polypeptide. Preferred ***Polynucleotide*** : (II) is chosen from a ***polynucleotide*** encoding a polypeptide having an amino acid ***sequence*** which has at least 99.1% identity with amino acids 1-411 of SEQ ID No. 4, and a ***polynucleotide*** having at least 98.9% identity with nucleotides 67-1299 of a fully defined 1299 nucleotide (SEQ ID No. 3) ***sequence*** given in the specification. Preferred Additive: (IV) further comprises one or more of amylase, additional phytase, xylanase, ***galactanase***, alpha-galactosidase, protease, phospholipase and/or beta-glucanase.

USE - (I) is useful in animal feed, in the preparation of a composition for use in animal feed, and for improving the nutritional value of an animal feed, where one or more of (I) is added to the feed. (claimed) and the improvement of nutritional value include promotion of growth of animal, improvement in feed utilization, improvement in bio-availability of proteins, increase of level of digestible phosphate, improvement of release and/or degradation of phytate, and improvement of bio-availability of trace minerals and macro minerals. (I) is useful in industrial applications for degradation of phytate, phytic acid and/or mono-, di-, tri-, tetra- and/or penta-phosphates of myoinositol, and for reducing phytate level in manure.

ADVANTAGE - (I) belonging to acid histidine phosphatase family, has high specificity towards the substrate phytate. (I) exhibits improved properties such as increased stability (acid-stability, heat-stability, protease stability and/or pepsin stability) at optimum pH, and improved performance in animal feed (such as improved release of phosphate and/or degradation of phytate).

EXAMPLE - The *Citrobacter braakii* ATCC 51113 phytase ***gene*** was cloned into the pET-30a(+) Escherichia coli expression vector without fusion tags. In the system, the expression of the ***gene*** was induced by providing a source of T7 RNA polymerase in the E. coli BL21 star(DE)pLysS host strain which contains a chromosomal copy of the T7 RNA polymerase ***gene*** under the control of the lacUV5 promoter. The induction of the target ***gene*** was performed by adding lactose to the media. Lactose will bind to the repressor and induce its dissociation from the operator, permitting transcription from the promoter. For expression of the phytase ***gene***, a single colony of the transformed E. coli strain was transferred into an inoculum culture in non-including media (containing glucose as the sole carbon source) that does not permit expression of the T7 RNA polymerase. As a negative control E. coli BL21 star(DE)pLysS containing an empty pET-30(+) vector was used. A small aliquot of the inoculum culture was transferred into flasks containing lactose as the sole carbon source. The induction culture was grown overnight with shaking at 300 rpm at 37degreesC. The cells were harvested by centrifugation, and 15 μl aliquots of the supernatant was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A distinct band of molecular weight of approximately 50 kDa was identified in the supernatant from the ***recombinant*** E. coli strain, but not in the negative control. Thus the ***recombinant*** phytase polypeptide was identified and isolated.(70 pages)

L5 ANSWER 7 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2006-11767 BIOTECHDS <<LOGINID::20080204>>

TITLE: Novel isolated polypeptide having phytase activity, useful in animal feed, and in preparation of composition for use in animal feed, for improving nutritional value of animal feed; involving vector-mediated gene transfer, expression in host cell, transgenic plant and transgenic animal model construction for use in feedstuff and reduced phytate level in excrement

AUTHOR: TAKAMIYA M; SJOHOLM C

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2006037327 13 Apr 2006

APPLICATION INFO: WO 2005-DK631 4 Oct 2005

PRIORITY INFO: DK 2004-1999 23 Dec 2004; DK 2004-1514 4 Oct 2004

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2006-293549 [30]

AN 2006-11767 BIOTECHDS <<LOGINID:20080204>>

AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide (I) having phytase activity, comprising an amino acid ***sequence*** having at least 75% identity with amino acids 1-409 of fully defined 431 amino acids (SEQ ID No. 2) sequences given in specification, amino acids 1-410 of fully defined 432 amino acids (SEQ ID No. 4) sequences given in specification, amino acids 1-414 of a fully defined 436 amino acids (SEQ ID No. 4) sequences given in specification, is new.

DETAILED DESCRIPTION - An isolated polypeptide (I) having phytase activity, chosen from: (a) a polypeptide having an amino acid

sequence which has at least 75% identity with amino acids 1-409 of a fully defined 431 amino acids (SEQ ID No. 2) sequences given in the specification, amino acids 1-410 of a fully defined 432 amino acids (SEQ ID No. 4) sequences given in the specification, amino acids 1-414 of a fully defined 436 amino acids (SEQ ID No. 4) sequences given in the specification, and their mature polypeptide portion of SEQ ID No. 2, 4 and 6; (b) a polypeptide which is encoded by a ***polynucleotide*** which hybridizes under at least medium stringency conditions with nucleotides 67-1293 of a fully defined 1296 nucleotides (SEQ ID No. 1)

sequence given in the specification, nucleotides 67-1296 of a fully defined 1296 nucleotides (SEQ ID No. 3) ***sequence*** given in the specification, nucleotides 67-1308 of a fully defined 1311 nucleotides (SEQ ID No. 5) ***sequence*** given in the specification, mature polypeptide encoding region of SEQ ID No. 1, 3 and 5, and complementary strand of above nucleotides; (c) a ***variant*** of any one of the above polypeptides comprising deletion, insertion, and/or conservative substitution of one or more amino acids; and (d) a fragment of any one of the above polypeptides. INDEPENDENT CLAIMS are also included for: (1) an isolated ***polynucleotide*** (II), comprising a nucleotide ***sequence*** which encodes (I); (2) an isolated

polynucleotide (III) encoding a polypeptide having phytase activity, chosen from a ***polynucleotide*** encoding a polypeptide having an amino acid ***sequence*** which has at least 75% identity with amino acids described in (I)(a), excluding the mature form, a

polynucleotide having at least 75% identity with nucleotides described in (I)(b), excluding the mature form, and a

polynucleotide which hybridizes under at least medium stringency conditions with nucleotides described in (I)(b); (3) a nucleic acid construct (III), comprising (II) operably linked to one or more control sequences that direct the production of the polypeptide in an expression host; (4) a ***recombinant*** expression vector (V1), comprising (III); (5) a ***recombinant*** host cell (H1), comprising (III); (6) producing (I); (7) a transgenic plant, or its parts or cell (P1), transformed with (II); (8) a transgenic, non-human animal, or its products or elements (A1), capable of expressing (I); (9) an animal feed additive (A2), comprising (I), and at least one fat soluble vitamin, at least one water soluble vitamin, and/or at least one trace mineral; and (10) an animal feed composition (C1) having a crude protein content of 50-800 g/kg and comprising (I).

BIOTECHNOLOGY - Preparation: (I) is produced by cultivating a cell, which in its wild-type form is capable of producing (I), under conditions conducive for production of (I), and recovering (I), or by cultivating (H1) under conditions conducive for production of (I), and recovering (I) (claimed). Preferred ***Polynucleotide*** : (II) has at least one mutation in the mature polypeptide coding ***sequence*** of SEQ ID No. 1, 3, or 5, in which the ***mutant*** nucleotide ***sequence*** encodes a polypeptide comprising amino acids 1-409 of SEQ ID No. 2, amino acids 1-410 of SEQ ID No. 4 or amino acids 1-414 of SEQ ID No. 6.

Preferred Additive: (A2) further comprises a amylase, an additional phytase, a xylanase, a ***galactanase***, a alpha-galactosidase, protease, phospholipase, and/or at beta-glucanase.

ACTIVITY - Anabolic. No biological data given.

MECHANISM OF ACTION - None given.

USE - (I) is useful in animal feed, and in the preparation of a composition for use in animal feed. (I) is useful for improving the nutritional value of an animal feed, when added to the feed (all

claimed). (I) is useful for reducing phytate level of manure, improving feed digestibility, promotes growth of animal, improving feed utilization, improving bio-availability of proteins, increases the level of digestible phosphate, improving release and/or degradation of phytate, improving bio-availability of trace minerals.

ADVANTAGE - (I) improves the nutritional value of an animal feed (claimed). (I) is acid-stable and has high specific activity.

EXAMPLE - The *Citrobacter gillenii* DSM 13694 phytase ***gene*** was cloned into the pET-30a(+) *Escherichia coli* expression vector without fusion tags. For expression of the phytase ***gene***, a single colony of the transformed *E. coli* strain was transferred into an inoculum culture in non-inducing media (containing glucose) that does not permit expression of the T7 RNA polymerase. A small aliquot (150 µl) of the inoculum culture was transferred into flasks containing lactose as the carbon source. The induction culture was grown overnight with shaking at 300 rpm at 37°C. The cells were harvested by centrifugation and 15 µl aliquots of the supernatant was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). *C. gillenii* DSM 13694 was grown overnight with shaking (225 rpm) at 30°C in LB medium with addition of 0.1% (w/w) sodium phytate. The cells were harvested by centrifugation (4000 rpm, 60 minutes) and the supernatant was discarded. The cell pellet was re-suspended in two volumes of distilled water with 100 mg/ml lysozyme and lysed by overnight incubation at 37°C. The lysed cells were centrifuged (4000 rpm, 2 h) and the supernatant saved and used for acid stability analysis.(68 pages)

L5 ANSWER 8 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2006-07472 BIOTECHDS <<LOGINID:20080204>>

TITLE: Microorganism expressed with enzymes capable of degrading polysaccharide such as cellulose, hemicellulose and starch, and enzymes capable of producing organic acid, on cell surface, useful for producing organic acid is new; polysaccharide degradation and degrading enzyme for use in lactic acid production

AUTHOR: TOKUHIRO T; TAKAHASHI H

PATENT ASSIGNEE: TOYOTA CHUO KENKYUSHO KK

PATENT INFO: JP 2006042719 16 Feb 2006

APPLICATION INFO: JP 2004-231062 6 Aug 2004

PRIORITY INFO: JP 2004-231062 6 Aug 2004; JP 2004-231062 6 Aug 2004

DOCUMENT TYPE: Patent

LANGUAGE: Japanese

OTHER SOURCE: WPI: 2006-159526 [17]

AN 2006-07472 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - Microorganism (I) capable of producing organic acid, using oligosaccharide or polysaccharide as carbon source, by expressing first ***polynucleotide*** encoding the degrading enzymes on the cell surface of microorganism, and transducing second ***polynucleotide*** encoding enzymes capable of producing organic acid, where the degrading enzymes degrades oligosaccharide chosen from cellulose, hemicellulose and starch, and monosaccharides, is new.

BIOTECHNOLOGY - Preferred Microorganism: In (I), the monosaccharides are glucose, galactose, mannose and fructose, and xylose and arabinose. The polysaccharide includes cellulose and hemicellulose. The polysaccharide having glucose structure, is linked by beta-1,4 linkages. The degrading enzymes include beta-glucosidase, endoglucanase and cellobiohydrolase. The oligosaccharide and polysaccharide has monosaccharides chosen from xylose, mannose, galactose, arabinose and their derivatives, and their respective degrading enzymes are beta-xylanase, beta-xylosidase, alpha-arabinofuranosidase, mannosidase, mannanase, arabinase, ***galactanase*** and pectinase. The first and second polynucleotides are foreign polynucleotides in the microorganism, and are provided on the host chromosome. (I) has alcohol fermentation ability. In (I), the PDC ***gene*** is disrupted. The second ***polynucleotide*** controls the promoter of PDC ***gene*** in host chromosome.

USE - (I) is useful for producing organic acid, which involves culturing (I) in a culture medium containing oligosaccharide or polysaccharide as a carbon source, and producing the organic acid. The organic acid is lactic acid, and the enzyme used is lactate

dehydrogenase. The carbon source is isolated from plants. The method utilizes amorphous cellulose or low molecular weight cellulose as a carbon source. The method further involves adding saccharides generated by the polysaccharides or oligosaccharides on reaction with degradation enzyme, as a partial carbon source. The saccharides are added at the time of initiating the cultivation process (all claimed).

ADVANTAGE - (I) can produce organic acid directly from the polysaccharide or oligosaccharide derived from plant.

EXAMPLE - The dihydrofolate reductase ***gene*** (RDHFR) derived from Escherichia coli was used as a selective marker. The total synthesis of the RDHFR ***gene*** was carried out using overlap PCR using synthetic DNA. The RDHFR marker cassette was built by connecting CYC1 promoter ***sequence*** and CYC1 terminator ***sequence*** with the upstream and downstream of a RDHFR ***gene***. The yeast genome DNA was made into template and the CCC1 ***gene*** downstream region ***sequence*** and RSA3 ***gene*** downstream region ***sequence***, which are homologous recombination ***sequence***, were amplified by PCR. The fragments CCC1-upstream and CCC1-downstream were digested by restriction enzymes SacI and SmaI, and represented as CCC1 fragment. The fragment RSA3-D digested by restriction enzymes ApaI and KpnI, represented as RSA3 fragment, and inserted to pFCBH 2W3, to obtain CBHII expression cassette. The fragment of CCC1 fragment, CBHII expression cassette, RDHFR marker cassette, and RSA3 fragment was connected to the multi-cloning site of a pBluescriptII SK+ cloning site of a pBluescriptII SK+ vector, to obtain pBRDHFR-TDJ3p-TrCBHII. The PDC1p-LDH was transduced on the chromosome of tryptophan requiring ***mutant*** yeast IF02260 strain. The transformant was selected using SD-Trp selective medium. The PDC1 structural ***gene*** was then disrupted by spore dissection operation, to produce microorganism capable producing organic acid.(21 pages)

L5 ANSWER 9 OF 31 USPATFULL on STN

ACCESSION NUMBER: 2005:144275 USPATFULL <<LOGINID::20080204>>

TITLE: Whole cell engineering by mutagenizing a substantial portion of a starting genome combining mutations and optionally repeating

INVENTOR(S): Short, Jay M, Rancho Santa Fe, CA, UNITED STATES
Fu, Pengcheng, Lowrey Avenue, HI, UNITED STATES
Wei, Jing, San Diego, CA, UNITED STATES
Levin, Michael, San Diego, CA, UNITED STATES
Latterich, Martin, Montellano Terrace, San Diego, CA,
UNITED STATES

NUMBER KIND DATE

PATENT INFORMATION: US 2005124010 A1 20050609
APPLICATION INFO.: US 2003-398271 A1 20011001 (10)
WO 2001-US31004 20011001

NUMBER DATE

PRIORITY INFORMATION: US 2000-9677584 20000930
US 2003-279702P 20010328 (60)

DOCUMENT TYPE: Utility

FILE SEGMENT: APPLICATION

LEGAL REPRESENTATIVE: FISH & RICHARDSON, PC, 12390 EL CAMINO REAL, SAN DIEGO,
CA, 92130-2081, US

NUMBER OF CLAIMS: 179

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 31 Drawing Page(s)

LINE COUNT: 31291

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to the field of cellular and whole organism engineering. Specifically, this invention relates to a cellular transformation, directed evolution, and screening method for creating novel transgenic organisms having desirable properties. Thus in one aspect, this invention relates to a method of generating a transgenic organism, such as a microbe or a plant, having a plurality of traits that are differentially activatable.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 10 OF 31 USPATFULL on STN
ACCESSION NUMBER: 2005:138016 USPATFULL <<LOGINID::20080204>>
TITLE: Methods for conducting assays for enzyme activity on
protein microarrays
INVENTOR(S): Zhou, Fang X., New Haven, CT, UNITED STATES
Schweitzer, Barry, Cheshire, CT, UNITED STATES

NUMBER KIND DATE

PATENT INFORMATION: US 2005118665 A1 20050602
APPLICATION INFO.: US 2004-865431 A1 20040609 (10)
RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 2003-458720, filed
on 9 Jun 2003, PENDING
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: JONES DAY, 222 EAST 41ST ST, NEW YORK, NY, 10017, US
NUMBER OF CLAIMS: 36
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 7 Drawing Page(s)
LINE COUNT: 6014

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods of conducting assays for enzymatic activity on microarrays useful for the large-scale study of protein function, screening assays, and high-throughput analysis of enzymatic reactions. The invention relates to methods of using protein chips to assay the presence, amount, activity and/or function of enzymes present in a protein sample on a protein chip. In particular, the methods of the invention relate to conducting enzymatic assays using a microarray wherein a protein and a substance are immobilized on the surface of a solid support and wherein the protein and the substance are in proximity to each other sufficient for the occurrence of an enzymatic reaction between the substance and the protein. The invention also relates to microarrays that have an enzyme and a substrate immobilized on their surface wherein the enzyme and the substrate are in proximity to each other sufficient for the occurrence of an enzymatic reaction between the enzyme and the substrate.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 11 OF 31 USPATFULL on STN
ACCESSION NUMBER: 2005:37355 USPATFULL <<LOGINID::20080204>>
TITLE: Identifying, monitoring, and sorting genetically
modified plant portions
INVENTOR(S): Winterboer, Denny C., Milford, IA, UNITED STATES
Thompson, Katie A., Hartley, IA, UNITED STATES

NUMBER KIND DATE

PATENT INFORMATION: US 2005032033 A1 20050210
APPLICATION INFO.: US 2004-872169 A1 20040618 (10)

NUMBER DATE

PRIORITY INFORMATION: US 2003-479923P 20030619 (60)
DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: JONES DAY, 222 EAST 41ST ST, NEW YORK, NY, 10017
NUMBER OF CLAIMS: 56
EXEMPLARY CLAIM: 1
LINE COUNT: 1444
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to compositions and methods for identifying, monitoring, and sorting specific genetically-modified plant portions from other genetically-modified plant portions. The present invention also relates to compositions and methods for identifying, monitoring, and sorting specific genetically-modified plant portions from non-genetically modified plant portions where both are present in a mixture. Either or both of the genetically modified plant portions or

the non-genetically modified plant portions can comprise a distinguishable marker which is identified and used for sorting such mixtures of plant portions. The present invention is also directed toward kits useful in the methods disclosed herein.

The compositions, methods, and kits of the present invention are used inter alia in high-throughput, sorting systems for identity preservation of a seed stock, to provide seed populations that are free of genetically-modified seeds, to isolate hybrid seed uncontaminated with selfed seed, and to isolate one type of genetically-modified plant portion from a mixture of genetically-modified plant portions.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 12 OF 31 USPATFULL on STN

ACCESSION NUMBER: 2005:77602 USPATFULL <<LOGINID::20080204>>

TITLE: Genes coding for tomato .beta.-galactosidase polypeptides

INVENTOR(S): Gross, Kenneth C., Ellicott City, MD, United States
Smith, David L., Columbia, MD, United States

PATENT ASSIGNEE(S): The United States of America as represented by the
Secretary of Agriculture, Washington, DC, United States
(U.S. government)

NUMBER KIND DATE

PATENT INFORMATION: US 6872813 B1 20050329

WO 9964564 19991216

APPLICATION INFO.: US 2000-701868 20001205 (9)
WO 1999-US12697 19990608

20001205 PCT 371 date

NUMBER DATE

PRIORITY INFORMATION: US 1998-88805P 19980609 (60)

DOCUMENT TYPE: Utility

FILE SEGMENT: GRANTED

PRIMARY EXAMINER: Bui, Phuong T.

ASSISTANT EXAMINER: Collins, Cynthia

LEGAL REPRESENTATIVE: Fado, John D., Graeter, Janelle S.

NUMBER OF CLAIMS: 10

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 20 Drawing Figure(s); 52 Drawing Page(s)

LINE COUNT: 2026

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Novel DNA sequences derived from a family of genes encoding .beta.-galactosidases in tomato are disclosed. .beta.-Galactosidase II has demonstrated enzyme activity in cell wall disassembly, leading to loss of tissue integrity and fruit softening. Modification of .beta.-galactosidase II gene expression in plants transformed for expression in the antisense direction results in improvement of the quality of fruit texture and firmness.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 13 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2006-03858 BIOTECHDS <<LOGINID::20080204>>

TITLE: New polypeptide having protease activity, useful in detergents or in animal feed or animal feed additives, in increasing digestible or soluble protein in animal feed, or in increasing the degree of hydrolysis of proteins in animal diets; vector-mediated protease gene transfer and expression in host cell for recombinant protein production and surfactant, animal feed or animal feed-additive manufacture

AUTHOR: LASSEN S F; SJOHOLM C; OSTERGAARD P R; FISCHER M

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2005123911 29 Dec 2005

APPLICATION INFO: WO 2005-DK396 17 Jun 2005

PRIORITY INFO: DK 2004-969 21 Jun 2004; DK 2004-969 21 Jun 2004

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2006-067502 [07]

AN 2006-03858 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide having protease activity, is new.

DETAILED DESCRIPTION - A new isolated polypeptide having protease activity comprising: (a) a polypeptide having an amino acid

sequence which has a degree of identity to amino acids 1-192 of the fully defined 383-amino acid ***sequence*** (SEQ ID NO: 6) of at least 71.5%; (b) a polypeptide which is encoded by a nucleic acid

sequence which hybridizes under very high stringency conditions with: (i) nucleotides 574-1149 of the fully defined 1152-amino acid

sequence (SEQ ID NO: 5); (ii) a subsequence of (i) of at least 100 nucleotides; and/or (iii) a complementary strand of (i) or (ii); (c)

a ***variant*** of the polypeptide having amino acids 1-192 of SEQ ID NO: 6 comprising a substitution, deletion, extension and/or insertion of one or more amino acids; (d) an allelic ***variant*** of (A), or (B); or (e) a fragment of (A), (B), or (D) that has protease activity.

INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid

sequence encoding the polypeptide having protease activity; (2) a nucleic acid construct comprising the nucleic acid ***sequence*** operably linked to one or more control sequences that direct the production of the polypeptide in a suitable expression host; (3) a

recombinant expression vector comprising the nucleic acid construct; (4) a ***recombinant*** host cell comprising the nucleic acid construct or vector; (5) a transgenic plant, or plant part, capable of expressing the polypeptide; (6) a transgenic, non-human animal, or its products or elements capable of expressing the polypeptide; (7) a method for producing a polypeptide which comprises cultivating the

recombinant host cell to produce a supernatant comprising the polypeptide and recovering the polypeptide; (8) a method for improving the nutritional value of an animal feed which comprises adding the protease to the feed; (9) an animal feed additive comprising the protease, fat-soluble vitamin, water-soluble vitamin, and/or trace mineral; (10) an animal feed having a crude protein content of 50 to 800 g/kg and comprising the protease; and (11) a method for the treatment of proteins.

BIOTECHNOLOGY - Preferred Polypeptide: The polypeptide comprises:

(1) amino acids 1-192 of the fully defined 383-amino acid ***sequence*** (SEQ ID NO: 6); (2) amino acids 1-192 of the fully defined 381-amino acid ***sequence*** (SEQ ID NO: 2); (3) amino acids 1-192 of the fully defined 383-amino acid ***sequence*** (SEQ ID NO: 4); or (4) amino acids 1-189 of the fully defined 384-amino acid

sequence (SEQ ID NO: 8). Preferred Nucleic Acid: The nucleic acid ***sequence*** encoding the polypeptide having protease activity

hybridizes under very high stringency conditions with: (i) nucleotides 574-1149 of SEQ ID NO: 5; (ii) a subsequence of (i) of at least 100 nucleotides; and/or (iii) a complementary strand of (i) or (ii) and/or has a degree of identity to nucleotides 574-1149 of SEQ ID NO: 5 of at least 79.3%. The nucleic acid ***sequence*** comprises: (a)

nucleotides 574-1149 of the fully defined 1152-bp ***sequence*** (SEQ ID NO: 3); (b) nucleotides 574-1149 of the fully defined 1152-bp

sequence (SEQ ID NO: 5); (c) nucleotides 586-1152 of the fully defined 1155-bp ***sequence*** (SEQ ID NO: 7); or (d) nucleotides

568-1143 of the fully defined 1146-bp ***sequence*** (SEQ ID NO: 1). The nucleic acid ***sequence*** is produced by: (a) hybridizing a DNA

under very high stringency conditions with: (i) nucleotides 574-1149 of SEQ ID NO: 5; (ii) a subsequence of (i) of at least 100 nucleotides; and/or (iii) a complementary strand of (i) or (ii); and (b) isolating the nucleic acid ***sequence***. Preferred Feed Additive: The animal feed additive further comprises amylase, phytase, xylanase,

galactanase, alpha-galactosidase, protease, phospholipase, and/or beta-glucanase. Preferred Method: The method for the treatment of proteins comprises adding the protease to at least one protein or protein source. The soybean is included among the at least one protein source.

Production (claimed): Producing the polypeptide comprises cultivating:

(i) Nocardiopsis dassonvillei subsp. dassonvillei DSM 43235; (ii) Nocardiopsis prasina DSM 15649; (iii) Nocardiopsis prasina DSM 14010; or

(iv) Nocardiopsis sp. DSM 16424 and recovering the polypeptide.

USE - The protease is useful in animal feed, in animal feed additives, in preparing a composition for use in animal feed, in improving the nutritional value of an animal feed, in increasing digestible and/or soluble protein in animal feed, in increasing the degree of hydrolysis of proteins in animal diets, in treating proteins, or in detergents (claimed). (90 pages)

L5 ANSWER 14 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-04899 BIOTECHDS <<LOGINID::20080204>>

TITLE: Novel polypeptide having alpha-amylase activity and/or carbohydrate-binding affinity, useful in preparing detergent composition and dough, and in textile desizing; for surfactant and ethanol preparation and pulping, textile and paper industry

AUTHOR: HOFF T; ANDERSEN C; SPENDLER T; PEDERSEN S; VIKSO-NIELSEN A; SCHAFER T; LIU J

PATENT ASSIGNEE: NOVOZYMES AS; NOVOZYMES NORTH AMERICA INC

PATENT INFO: WO 2005001064 6 Jan 2005

APPLICATION INFO: WO 2004-US23031 25 Jun 2004

PRIORITY INFO: US 2003-519554 12 Nov 2003; DK 2003-949 25 Jun 2003

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2005-075552 [08]

AN 2005-04899 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - A polypeptide (I) having alpha-amylase activity and/or carbohydrate-binding affinity, comprising amino acid ***sequence*** having at least 90% identity with amino acid residues at position 1-586 of a fully defined ***sequence*** (S1) of 619 amino acids as given in the specification, or having at least 96% identity with amino acid residues at position 1-542 of a fully defined ***sequence*** (S2) of 575 amino acids as given in the specification, is new.

DETAILED DESCRIPTION - A polypeptide (I) having alpha-amylase activity and/or carbohydrate-binding affinity, chosen from: (a) polypeptide (P1) comprising an amino acid ***sequence*** having at least 90% identity with amino acid residues at position 1-586 of a fully defined ***sequence*** (S1) of 619 amino acids as given in the specification, a polypeptide (P2) comprising an amino acid ***sequence*** having at least 96% identity with amino acid residues at position 1-542 of a fully defined ***sequence*** (S2) of 575 amino acids as given in the specification, a polypeptide (P3) comprising an amino acid ***sequence*** having at least 80% identity with amino acid residues at position 1-583 of a fully defined ***sequence*** (S3) of 613 amino acids as given in the specification, a polypeptide (P4) encoded by a nucleotide ***sequence*** that hybridizes under high stringency conditions with a ***polynucleotide*** probe having the complementary strand of nucleotides 100-1857 of a fully defined ***sequence*** (S4) of 1860 base pairs as given in the specification, nucleotides 100-1725 of a fully defined ***sequence*** (S5) of 1728 base pairs as given in the specification or nucleotides 91-1839 of a fully defined ***sequence*** (S6) of 1842 base pairs as given in the specification, or a fragment of any one of P1-P4 that has alpha-amylase activity; and (b) polypeptide (P5) comprising an amino acid ***sequence*** having at least 60% identity with amino acid residues at position 485-586, 485-542 or 455-583 of (S1), (S2) or (S3), a polypeptide (P6) encoded by a nucleotide ***sequence*** that hybridizes under high stringency conditions with a ***polynucleotide*** probe having the complementary strand of nucleotides 1552-1857, 1552-1725 or 1453-1839 of (S4), (S5) or (S6), or a fragment of P5 or P6 that has carbohydrate binding affinity. INDEPENDENT CLAIMS are also included for the following:

(1) a ***polynucleotide*** (II) having a nucleotide ***sequence*** encoding (I); (2) a nucleic acid construct (III) comprising the nucleotide ***sequence*** encoding (I), operably linked to one or more control sequences that direct the production of the polypeptide in a suitable host; (3) a ***recombinant*** expression vector (V1) comprising (III); (4) a ***recombinant*** host cell (IV) comprising (III) or (V1); (5) producing (I); (6) a composition (C1) comprising (I); (7) cleaning or detergent composition (C2), preferably a laundry or dish wash composition, comprising (I), and further comprising a surfactant;

and (8) dough (V) comprising (I).

BIOTECHNOLOGY - Preparation: (I) is produced by cultivating (IV) under conditions conductive for production of the polypeptide, or cultivating a strain, which in its wild-type form is capable of producing the polypeptide to produce the polypeptide, and recovering the polypeptide. Preferred Polypeptide: In (I), the P1 comprises an amino acid ***sequence*** which as at least 90% identity with amino acid residues at position 1-586 of (S1), preferably at least 91%, 92%, 93%, 94%, 95% or 96% identity, more preferably at least 97%, 98% or 99% identity with amino acid residues at position 1-586 of (S1). P2 comprises an amino acid ***sequence*** having at least 96% identity with amino acid residues at position 1-542 of (S2), preferably at least 97% identity, more preferably 98% or 99% identity with amino acid residues at position 1-542 of (S2). P3 comprises an amino acid ***sequence*** which has at least 80% identity with amino acid residues 1-583 of S3, preferably at least 85%, 90%, 95% or 96% identity, more preferably at least 97%, 98% or 99% identity with amino acid residues position 1-583 of (S3). (I) having alpha-amylase activity, comprises the amino acid residues at position 1-586 of (S1), 1-542 of (S2), 1-583 of (S3), preferably 1-484 of (S1), 1-484 of (S2) or 1-455 of (S3). (I) is an artificial ***variant*** which comprises an amino acid ***sequence*** that has at least one substitution, deletion and/or insertion of an amino acid as compared to amino acid residues at position 1-586 of (S1), 1-542 of (S2) or 1-583 of (S3), respectively. P1 comprises an amino acid ***sequence*** which has at least 90% identity with the polypeptide encoded by the alpha-amylase encoding portion of the DNA ***sequence*** shown at position 100-1857 of (S4), preferably at least 91-98% or 99% identity with the polypeptide encoded by the alpha-amylase encoding DNA ***sequence*** shown at position 100-1857 of (S4). P2 comprises an amino acid ***sequence*** which has at least 96% identity with the polypeptide encoded by the alpha-amylase encoding portion of the DNA ***sequence*** shown at position 100-1725 of (S5), preferably at least 97%, 98% or 99% identity with the polypeptide encoded by the alpha-amylase encoding portion of the DNA ***sequence*** shown at position 100-1725 of (S5). P3 comprises an amino acid ***sequence*** which has at least 80% identity with the polypeptide encoded by the alpha-amylase encoding portion of the DNA ***sequence*** shown at position 91-1839 of (S6), preferably at least 85%, 90%, 91-98% or 99% identity with the polypeptide encoded by the alpha-amylase encoding DNA ***sequence*** shown at position 91-1839 of (S6). (I) is an artificial ***variant*** which comprises an amino acid ***sequence*** that has at least one substitution, deletion and/or insertion of an amino acid as compared to the amino acid ***sequence*** encoded by the alpha-amylase encoding portion of the DNA ***sequence*** shown at position 100-1857 of (S4) or 100-1725 of (S5) or 91-1839 of (S6). The carbohydrate-binding affinity is starch-binding affinity. (I) having carbohydrate-binding affinity, comprises an amino acid ***sequence*** which has at least 70% identity with amino acid residues at position 485-586 of (S1), preferably at least 80%, 85%, 90%, 95%, 96% or 97%, more preferably at least 99% identity with amino acid residues at position 485-586 of (S1). (I) having carbohydrate-binding affinity comprises the amino acid residues at position 485-586 of (S1), 485-542 of (S2), or 485-583 of (S3). (II) having carbohydrate-binding affinity comprises an amino acid ***sequence*** which has at least 70% identity with amino acid residues at position 485-586 of (S1), 485-542 of (S2), or 485-583 of (S3), preferably at least 80%, 85%, 90%, 95% or 96% identity, and more preferably at least 97%, 98% or 99% identity with amino acid residues at position 485-586 of (S1), 485-542 of (S2), or 485-583 of (S3). (I) having carbohydrate-binding affinity is an artificial ***variant*** that comprises an amino acid ***sequence*** that has at least one substitution, deletion and/or insertion of an amino acid as compared to amino acid residues at position 485-586 of (S1), 485-542 of (S2) or 485-583 of (S3). (I) having carbohydrate-binding affinity comprises an amino acid ***sequence*** which has at least 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity with the polypeptide encoded by the DNA ***sequence*** shown at position 1552-1857 of (S4), 1552-1725 of (S5), or 1453-1839 of (S6), respectively. (I) having carbohydrate-binding affinity is an artificial ***variant*** which comprises an amino acid ***sequence*** that has at least one substitution, deletion and/or insertion of an amino acid as compared to amino acid ***sequence***

encoded by the carbohydrate-binding domain encoding portion of the DNA ***sequence*** shown in position 1552-1857 of (S4), 1552-1725 of (S5) or 1453-1839 of (S6). (I) having carbohydrate-binding affinity is encoded by a DNA ***sequence*** that hybridizes under medium stringency conditions, preferably under high stringency conditions, with a

polynucleotide probe chosen from complementary strand of nucleotides 1552-1857, 1552-1725 or 1453-1839 of (S4), (S5) or (S6). Preferred Cell: (C1) or (C2) additionally comprises one or more enzymes chosen from cellulase such as an endoglucanase, lipase, cutinase, oxidoreductase, protease, another amylase, hemicellulase such as mannanase, xylanase, ***galactanase***, arabinofuranosidase, esterase, lichenase, arabinanases, pectate lyase and their mixture.

USE - (I) is useful in cleaning or detergent composition, preferably laundry or dish wash composition. (I) is useful for desizing textiles, which involves treating the textile with (I), and for preparing a dough-based product, which involves adding (I) and a xylanase. (I) is useful for preparing an edible product, which involves adding (I) to a dough, leavening, and heating the dough. (I) is useful for preparing dough or a baked product made from the dough, which involves adding (I). (I) or C1 is useful for treating textiles, fabrics, yarn or garments, in dough, for improving the elasticity of breadcrumb of a baking product, for improving the firmness of breadcrumb of a baking product, for improving the softness of breadcrumb of a baking product, and for improving the moistness of a baking product. (I) or C1 is useful for liquefaction of starch, and in ethanol production (all claimed). (I) or C1 is useful in beer making or brewing, pulp and paper production, production of sweeteners, ethanol and other fermentation products, especially fuel, industrial ethanol from e.g. starch or whole grains.

ADVANTAGE - (I) enables alpha-amylase activity and/or carbohydrate-binding affinity.

EXAMPLE - No relevant example is given. (79 pages)

L5 ANSWER 15 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
DUPLICATE 2

ACCESSION NUMBER: 2004-17744 BIOTECHDS <<LOGINID::20080204>>

TITLE: Novel variant of parent glycoside hydrolase family 53

galactanase, useful in dairy industry, to prepare galacto-oligosaccharide and/or for hydrolysis of lactose; recombinant enzyme production via plasmid expression in host cell for use in dairy product

AUTHOR: DE MARIA L; SVENDSEN A; BORCHERT T V; CHRISTENSEN L L H;
LARSEN S; RYTTERSGAARD C

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2004056988 8 Jul 2004

APPLICATION INFO: WO 2003-DK851 11 Dec 2003

PRIORITY INFO: DK 2003-537 8 Apr 2003; DK 2002-1968 20 Dec 2002

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2004-507720 [48]

AN 2004-17744 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - A ***variant*** of parent glycoside hydrolase family 53 ***galactanase*** having ***galactanase*** activity (I), comprising alteration at one of 248 positions such as -6, -4, -2, 1, 3, 4, 5, 6, 7, etc, where the alteration(s) are independently an insertion of amino acid immediately downstream of position, deletion of amino acid which occupies position, and/or substitution of amino acid which occupies position, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid ***sequence*** (II), comprising nucleic acid ***sequence*** encoding (I); (2) a nucleic acid construct (III) comprising (II), operably linked to one or more control sequences that direct the production of the ***galactanase*** ***variant*** in a suitable expression host; (3) ***recombinant*** expression vector (IV) comprising (III); (4) a ***recombinant*** host cell (V) comprising (III) or (IV); and (5) producing (I).

BIOTECHNOLOGY - Preparation: (I) is produced by culturing (IV), and recovering (I) from the culture medium (claimed). Preferred

Variant : (I) is a ***variant*** of *Myceliophthora thermophila* ***galactanase***. (I) comprises substitutions such as -6Pro; -4Pro;

-2Pro; 1Pro; 3Pro; 5Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 6Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 7Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 10Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 11Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 12Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 13Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 14Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 15Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 16Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 18Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 20Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 22Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 24Cys, Pro, 25Pro, 26Pro, 29Pro, 30Cys, 31Pro, 32Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 36Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 39Cys, 40Cys, 41Pro, 43Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 44Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 45Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 46Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 47Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 48Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 51Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 52Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 53Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 54Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 54fAla, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 54gAla, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 54hAla, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 55Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 56Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 57Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 58Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 61Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 62Cys, 65Cys, 69Cys, 77Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 79Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 80Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 81Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; 82Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; etc. Preferably, the substitutions includes Tyr214Asn, Ser+Asn247Tyr+Leu306Gln; Tyr214Ala; Phe216Phe, Val, Ala, Ser, Thr, Gly; and/or Pro89Trp+Trp86Asn; Ala90Ser+His91Asp; His91Asn, Leu, Asp; Asn313Asp; Asn303Asp, His; and/or Asn305Asp, His; Tyr22Pro, Asn24Pro, Thr25Pro, Ala29Pro, Ala29Pro, Ala53Pro, Asn56Pro, Thr93Pro, Asp101Pro, Trp142Pro, Thr147, Gln198Pro, Leu203Pro, Ser204Pro, Ser219Pro, Ser258Pro, Ser288Pro, Ala304Pro, Ala311Pro, Gln318Pro, Ala322Pro, Ser324Pro, Ser325Pro, and/or Ser327Pro; Trp107Ser, His; Gln126Glu; Asn39Cys+Leu326Cys; Val20Cys+Gly320Cys; Tyr110Cys+Gly163Cys; Trp150Cys+Asn194Cys; Thr274Cys+Val328Cys; and/or Ile301Cys+Phe316Cys;

and/or Gly6Ala, Cys, Asp, Glu, Phe, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Val7Ala, Cys, Asp, Glu, Phe, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Trp, Tyr; Asp8Ala, Cys, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Trp9Ala, Cys, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Tyr; Ser10Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Tyr; Ser11Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Thr, Val, Trp, Tyr; Val12Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Trp, Tyr; Val13Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Thr, Trp, Tyr; Val14Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Trp, Tyr; Glu15Ala, Cys, Asp, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Glu16Ala, Cys, Asp, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr, etc. (I) is a ***variant*** of *Humicola insolens* ***galactanase***, and comprises asterisk-6Pro; asterisk-4Pro; asterisk-2Pro; Ala1Pro; Gln3Pro; Lys5Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Gly6Ala, Cys, Asp, Glu, Phe, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Asp7Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Trp8Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Tyr; Trp9Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Tyr; Ser10Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Ser11Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Thr, Val, Trp, Tyr; Val12Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Trp, Tyr; Met13Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr, etc. (I) is a ***variant*** of *Aspergillus aculeatus* ***galactanase*** comprising amino acid substitution such as asterisk-6Pro; asterisk-4Pro; asterisk-2Pro; Ala1Pro; Thr3Pro; Arg5Ala, Cys, Asp, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, PRO, Gln, Ser, Thr, Val, Trp, Tyr; Gly6Ala, Cys, Asp, Glu, Phe, His, Ile, Lys, Leu, Met, Asn, PRO, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Ala7Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Asp8Ala, Cys, Glu, Phe, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Ile9Ala, Cys, Asp, Glu, Phe, Gly, His, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr, etc. (I) is a ***variant*** of *Bacillus licheniformis* ***galactanase*** comprising amino acid substitution such as Lys-6Pro; Ser-4Pro; Leu-2Pro; Lys1Pro; Phe3Pro; Lys5Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Gly6Ala, Cys, Asp, Glu, Phe, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Val7Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Trp, Tyr; Asp8Ala, Cys, Asp, Glu, Phe, Gly, His, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Val, Trp, Tyr; Val9Ala, Cys, Asp, Glu, Phe, Gly, His, Ile, Lys, Leu, Met, Asn, Pro, Gln, Arg, Ser, Thr, Trp, Tyr, etc. The number designating each position corresponds to the amino acid residue of *Myceliophthora thermophila* ***galactanase*** (MTGAL) and from the parent ***galactanase***. The parent ***galactanase*** has 25% ***sequence*** identity with *Myceliophthora thermophila* ***galactanase***. The parent ***galactanase*** is obtained from strains of *Yersinia*, *Aspergillus*, *Humicola*, *Meripilus*, *Myceliophthora* or *Thermomyces*, or *Bacillus*, *Bifidobacterium*, *Cellvibrio*, *Clostridium*, *Pseudomonas*, *Thermotoga* or *Xanthomonas*.
 USE - (I) is useful in dairy industry, to prepare galacto-oligosaccharide and/or for hydrolysis of lactose (claimed).
 EXAMPLE - The ***galactanase*** variants were prepared as follows. The Asp181Asn mutation was introduced in to *Aspergillus aculeatus* ***galactanase*** (AAGAL) encoding ***gene*** by the use of the mutagenic oligonucleotide 5'-CATTTG GACAACGGCTGGAGC-3'. The mutations Asp181Asn+Ser90Ala+Asp91His were introduced in a similar way. The resulting ***variant*** genes were cloned into plasmid pHD464 as described in Dalboe H., Heldt-Hansen H. 1994. The Ala90Ser+His91Asp double mutation was introduced in the *Myceliophthora thermophila* ***galactanase*** (MTGAL) encoding ***gene*** essentially as described above by the use of the mutagenic oligonucleotide 5'-GCCGATCCTCTGATCAGACCATGCC-3'. Proteins were expressed in and secreted

from Aspergillus oryzae as described in christensen, T., Woldike, H., Boel, E., Mortensen, S.B., Hjortshoj, K., Thim, L., Hansen, M.T., 1998. The culture supernatant from a fermentation of the A.oryzae strain was filtered through a 0.22 microm filter to remove the mycelia. About 1200 ml filtrate was added ammonium sulfate to a concentration of 1.6 M, loaded onto a 50 ml butyl column equilibrated with 25 mM sodium acetate, 1.6 M ammonium sulfate (pH 5.0) and eluted using a linear ammonium sulfate decreasing from 1.6 M to 0 M over 10 column volumes.

Galactanase activity was measured by mixing 40 microl of fractions with lupin AZCL-galactan (200 microl, 10 mg/ml) in 0.5 M MES pH 6.5. After 30 minutes incubation at room temperature, insoluble substrate was removed by centrifugation, and absorbance of supernatant measured at 590 nm. Fractions containing ***galactanase*** activity eluted around 1 M ammonium sulfate were pooled and dialyzed against 10 mM sodium citrate (pH 3.5). Dialysate (400 ml) was dilute to 2000 ml with water and loaded onto a 50 ml S-Sepharose column equilibrated with 10 mM sodium citrate pH 3.5. The concentrate obtained was 95% pure. (262 pages)

L5 ANSWER 16 OF 31 USPATFULL on STN

ACCESSION NUMBER: 2004:101228 USPATFULL <<LOGINID::20080204>>

TITLE: Whole cell engineering by mutagenizing a substantial portion of a starting genome, combining mutations, and optionally repeating

INVENTOR(S): Short, Jay M., Rancho Santa Fe, CA, UNITED STATES

NUMBER KIND DATE

PATENT INFORMATION: US 2004077090 A1 20040422

APPLICATION INFO.: US 2003-383798 A1 20030306 (10)

RELATED APPLN. INFO.: Continuation of Ser. No. US 2000-677584, filed on 30 Sep 2000, ABANDONED Continuation-in-part of Ser. No. US 2000-594459, filed on 14 Jun 2000, GRANTED, Pat. No. US 6605449 Continuation-in-part of Ser. No. US 2000-522289, filed on 9 Mar 2000, GRANTED, Pat. No. US 6358709 Continuation-in-part of Ser. No. US 2000-498557, filed on 4 Feb 2000, PENDING Continuation-in-part of Ser. No. US 2000-495052, filed on 31 Jan 2000, GRANTED, Pat. No. US 6479258

NUMBER DATE

PRIORITY INFORMATION: US 1999-156815P 19990929 (60)

DOCUMENT TYPE: Utility

FILE SEGMENT: APPLICATION

LEGAL REPRESENTATIVE: HALE AND DORR LLP, 300 PARK AVENUE, NEW YORK, NY, 10022

NUMBER OF CLAIMS: 22

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 28 Drawing Page(s)

LINE COUNT: 37121

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB An invention comprising cellular transformation, directed evolution, and screening methods for creating novel transgenic organisms having desirable properties. Thus in one aspect, this invention relates to a method of generating a transgenic organism, such as a microbe or a plant, having a plurality of traits that are differentially activatable. Also, a method of retooling genes and gene pathways by the introduction of regulatory sequences, such as promoters, that are operable in an intended host, thus conferring operability to a novel gene pathway when it is introduced into an intended host. For example a novel man-made gene pathway, generated based on microbially-derived progenitor templates, that is operable in a plant cell. Furthermore, a method of generating novel host organisms having increased expression of desirable traits, recombinant genes, and gene products.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 17 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN

ACCESSION NUMBER: 2005-03101 BIOTECHDS <<LOGINID::20080204>>

TITLE: New secreted mature polypeptide with protease activity, useful for increasing digestible or soluble protein or degree

of hydrolysis of protein in animal diet, for treating vegetable protein or as a component of a detergent composition;
vector-mediated secreted enzyme gene transfer and expression in host cell for recombinant protein production and animal feed composition manufacture

AUTHOR: LASSEN S F

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2004111224 23 Dec 2004

APPLICATION INFO: WO 2004-DK436 21 Jun 2004

PRIORITY INFO: DK 2004-334 1 Mar 2004; DK 2003-916 19 Jun 2003

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2005-040104 [04]

AN 2005-03101 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - A secreted mature polypeptide having protease activity after maturation, is new.

DETAILED DESCRIPTION - The new secreted mature polypeptide having protease activity after maturation comprises: (1) a ***sequence*** having at least 70% identity with the mature part of the polypeptide ***sequence*** comprising 353 (each of the 3 sequences), 354, 355 or 388 amino acids or encoded by the ***polynucleotide*** ***sequence*** comprising 1062 (each of the 3 sequences), 1143, 1112, 1146, 1068 or 1164 bp; (2) a ***variant*** of the mature part of the polypeptide ***sequence*** comprising 353 (each of the 3 sequences), 354, 355 or 388 amino acids, having a substitution, deletion, extension and/or insertion of one or more amino acids; (3) an allelic ***variant*** of (1), (2) or (3); or (4) a fragment of (1), (2), (3) or (4). The polypeptide comprises a heterologous pro-region when expressed and before maturation. INDEPENDENT CLAIMS are also included for the following: (1) an isolated ***polynucleotide*** encoding the polypeptide; (2) a ***recombinant*** expression vector or ***polynucleotide*** construct comprising the ***polynucleotide*** ; (3) a ***recombinant*** host cell comprising the ***polynucleotide*** , expression vector or ***polynucleotide*** construct; (4) a transgenic plant or its part comprising the ***polynucleotide*** , expression vector or ***polynucleotide*** construct; (5) a transgenic, non-human animal or its products or elements comprising the ***polynucleotide*** , expression vector or ***polynucleotide*** construct; (6) a method for producing the polypeptide; (7) an animal feed additive comprising the polypeptide and at least one fat-soluble vitamin, water-soluble vitamin or trace mineral; (8) an animal feed composition having a crude protein content of 50-800 g/kg and comprising the polypeptide or feed additive; (9) a composition comprising the polypeptide and at least one enzyme; and (10) a method for using the polypeptide for improving the nutritional value of an animal feed, for increasing digestible or soluble protein in animal diets, for increasing the degree of hydrolysis of proteins in animal diets or for treating vegetable proteins, or as a component of a detergent composition.

BIOTECHNOLOGY - Preferred Polypeptide: The polypeptide is a wild-type polypeptide or its artificial ***variant*** having one or more non-polar or uncharged amino acids comprising Gln-Ser-His-Val-Gln-Ser-Ala-Pro, Gln-Ser-Ala-Pro, Gln-Pro, Thr-Leu, Thr-Thr, Gln-Leu, Thr-Pro, Leu-Pro, Thr-Ile, Ile-Gln, Gln-Pro, Pro-Ile, Leu-Thr, Thr-Gln, Ile-Thr, Gln-Gln or Pro-Gln, added to the C-terminus as compared to the wild-type, a shuffled polypeptide or a protein-engineered polypeptide. The polypeptide comprises at least three non-polar or uncharged polar amino acids within the last four amino acids of the C-terminus of the polypeptide. When expressed and before maturation, the polypeptide comprises a heterologous secretion signal-peptide that is cleaved from the polypeptide when the polypeptide is secreted or that is derived from a heterologous protease. The heterologous secretion signal-peptide comprises a ***sequence*** having at least 70% identity with the amino acid ***sequence*** encoded by polynucleotides 1-81 of the ***sequence*** comprising 1143 or 1164 bp. The heterologous pro-region is derived from S2A or S1E protease or is preferably, at least 70% identical to the pro-region of the polypeptide ***sequence*** comprising 353 (each of the 3 sequences), 166 (each of the 6 sequences),

165, 354, 355 or 388 amino acids. Preferred Host Cell: The ***recombinant*** host cell is a Bacillus cell. Preferred Composition: The composition comprises the polypeptide and at least one enzyme comprising phytase (EC 3.13.8 OR 3.13.26), xylanase (EC 3.2.1.8), ***galactanase*** (EC 3.2.1.89), alpha-galactosidase (EC 3.2.1.22), protease (EC 3.4.--), phospholipase A1 (EC 3.1.1.32), phospholipase A2 (EC 3.1.1.4), lysophospholipase (EC 3.1.1.5), phospholipase C (3.1.4.3), phospholipase D (EC 3.1.4.4) or beta-glucanase (EC 3.2.1.4 or EC3.2.1.6). Preferred Method: Using the polypeptide for improving the nutritional value of an animal feed, for increasing digestible or soluble protein in animal diets, for increasing the degree of hydrolysis of proteins in animal diets or for treating vegetable proteins comprises including the polypeptide in animal feed or in a composition for use in animal feed. Using the polypeptide as a component of a detergent composition comprises including the polypeptide in a detergent composition. Production (claimed): Producing the polypeptide comprises cultivating the ***recombinant*** host cell or transgenic plant or animal to produce a supernatant comprising the polypeptide and optionally recovering the polypeptide.

USE - The polypeptide is useful for improving the nutritional value of an animal feed, for increasing digestible or soluble protein in animal diets, for increasing the degree of hydrolysis of proteins in animal diets or for treating vegetable proteins, or as a component of a detergent composition (claimed).

EXAMPLE - No relevant examples given.(121 pages)

L5 ANSWER 18 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-03100 BIOTECHDS <<LOGINID::20080204>>

TITLE: Novel isolated polypeptide having protease activity, useful in animal feed and for improving nutritional value of animal feed;
vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production and animal feed composition manufacture

AUTHOR: LASSEN S F; SJOHOLM C; OSTERGAARD P R

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2004111223 23 Dec 2004

APPLICATION INFO: WO 2004-DK435 21 Jun 2004

PRIORITY INFO: DK 2003-915 19 Jun 2003; DK 2003-915 19 Jun 2003

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2005-040103 [04]

AN 2005-03100 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide (I) having protease activity, comprising an amino acid ***sequence*** with degree of identity to amino acids at positions 1-188 of a fully defined ***sequence*** (S1) of 353 amino acids as given in the specification, of at least 99.0%, is new.

DETAILED DESCRIPTION - An isolated polypeptide (I) having protease activity, is chosen from polypeptide (P1) comprising an amino acid ***sequence*** which has a degree of identity to amino acids at positions 1-188 of a fully defined ***sequence*** (S1) of 353 amino acids as given in the specification of at least 99.0 %; a polypeptide (P2) encoded by a nucleic acid ***sequence*** which hybridizes under medium-high stringency conditions with nucleotides (N1) at positions 496-1059 of a fully defined ***sequence*** (S2) of 1062 base pairs as given in the specification, a subsequence of N1 of at least 100 nucleotides, and/or a complementary strand of N1 or its subsequence of at least 100 nucleotides; a ***variant*** (P3) of the polypeptide having an amino acid ***sequence*** of amino acids 1-188 of (S1) comprising a substitution, deletion, extension, and/or insertion of one or more amino acids; an allelic ***variant*** (P4) of P1, P2 or P3, and a fragment of any one of P1-P4 having protease activity. INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid ***sequence*** (II) comprising a nucleic acid ***sequence*** : (a) encoding (I); (b) encoding a polypeptide having protease activity, and hybridizing under medium-high stringency conditions with N1, its subsequence of at least 100 nucleotides and/or a complementary strand of N1 or its subsequence; and/or (c) encoding a polypeptide having protease activity and having a

degree of identity to N1 of at least 97.8 %; (2) a nucleic acid construct (III) comprising (II) operably linked to control sequences that direct the production of the polypeptide in a suitable expression host; (3) a ***recombinant*** expression vector (V1) comprising (III); (4) a ***recombinant*** host cell (IV) comprising (III) or V1; (5) producing (I); (6) a transgenic plant, or plant part, capable of expressing (I); (7) a transgenic, non-human animal, or its products or elements, being capable of expressing (I); (8) an animal feed additive (V) comprising (I), and one or more fat-soluble vitamin, water-soluble vitamin, and/or trace minerals; (9) an animal feed composition having a crude protein content of 50-800 g/kg and comprising (I), or at least one (V); (10) a composition comprising (I), together with at least one other enzyme chosen from phytase (EC 3.1.3.8 or 3.1.3.26), xylanase (EC 3.2.1.8),

galactanase (EC 3.2.1.89), alpha-galactosidase (EC 3.2.1.22), protease (EC 3.4.-.-), phospholipase A1 (EC 3.1.1.32), phospholipase A2 (EC 3.1.1.4), lysophospholipase (EC 3.1.1.5), phospholipase C (EC 3.1.4.3), phospholipase D (EC 3.1.4.4), amylase and/or beta-glucanase (EC 3.2.1.4 or EC 3.2.1.6); and (11) Nocardiopsis prasina DSM 15649.

BIOTECHNOLOGY - Preparation: (I) is produced by cultivating (IV) to produce a supernatant comprising (I) and recovering (I) (claimed).

USE - (I) is useful in animal feed, in the preparation of a composition for use in animal feed, for improving the nutritional value of an animal feed, for increasing digestible and/or soluble protein in animal diets, for increasing the degree of hydrolysis of proteins in animal diets, and/or for the treatment of proteins. (I) is useful in detergents. (All claimed.)

ADVANTAGE - (I) is a low-allergenic ***variant***, capable of invoking reduced immunological response when exposed to animals.

EXAMPLE - Genomic DNA from Nocardiopsis prasina DSM 15649 was isolated, and used as template for PCR amplification using primers having sequences such as 5'-gttcatcgatcgcatggctggccacggactccccagtc-3' and 5'-gcggatcctttaggtccggagacggacgcggccaggag-3'. The PCR fragment was isolated on agarose gel (0.7 %). The digested and purified PCR fragment was ligated to the Clal and BamHI digested plasmid pDG268NeoMCS-PramyQ/PrcryIII/cryIIIAstab/Sav. The ligation mixture was used for transformation into Escherichia coli TOP10F' and several colonies were selected for miniprep. The purified plasmids were checked for insert before transformation into a strain of Bacillus subtilis derived from B. subtilis DN1885 with disrupted apr, npr and pel genes. The transformed host cells were fermented on a rotary shaking table in baffled Erlenmeyer flasks (500 ml) containing PS-1 medium (100 ml) supplemented with chloramphenicol (6 microg/ml), at 37 degreesC for 16 hours and at 26 degreesC for extra 4 days. The culture broth was centrifuged and supernatants were carefully decanted from the precipitates. The combined supernatants were filtered through a Seitz EKS plate to remove the rest of the Bacillus host cells. The EKS filtrate was applied to a bacitracin silica column, then to G25 Sephadex column and to S sepharose HP column, from which fractions containing protease were eluted. (60 pages)

L5 ANSWER 19 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-03099 BIOTECHDS <<LOGINID::20080204>>

TITLE: Novel isolated polypeptide having protease activity, useful in animal feed and for improving nutritional value of animal feed;

vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production and animal feed composition manufacture

AUTHOR: LASSEN S F; SJOHOLM C; OSTERGAARD P R

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2004111222 23 Dec 2004

APPLICATION INFO: WO 2004-DK434 21 Jun 2004

PRIORITY INFO: DK 2003-914 19 Jun 2003; DK 2003-914 19 Jun 2003

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2005-040102 [04]

AN 2005-03099 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide (I) having protease activity, comprising an amino acid ***sequence*** with degree of identity to amino acids at positions 1-188 of a fully defined ***sequence*** (S1)

of 353 amino acids as given in the specification, of at least 98.0 %, is new.

DETAILED DESCRIPTION - An isolated polypeptide (I) having protease activity, is chosen from polypeptide (P1) comprising an amino acid

sequence which has a degree of identity to amino acids at positions 1-188 of a fully defined ***sequence*** (S1) of 353 amino acids as given in the specification of at least 98.0 %; a polypeptide (P2) encoded by a nucleic acid ***sequence*** which hybridizes under medium-high stringency conditions with nucleotides (N1) at positions 496-1059 of a fully defined ***sequence*** (S2) of 1062 base pairs as given in the specification, a subsequence of N1 of at least 100 nucleotides, and/or a complementary strand of N1 or its subsequence, of at least 100 nucleotides; a ***variant*** (P3) of the polypeptide having an amino acid ***sequence*** of amino acids 1-188 of (S1) comprising a substitution, deletion, extension, and/or insertion of one or more amino acids; an allelic ***variant*** (P4) of P1, P2 or P3, and a fragment of any one of P1-P4 having protease activity. INDEPENDENT CLAIMS are also included for: (1) an isolated nucleic acid

sequence (II) comprising a nucleic acid ***sequence*** : (a) encoding (I); (b) encoding a polypeptide having protease activity, and hybridizing under medium-high stringency conditions with N1, its subsequence of at least 100 nucleotides and/or a complementary strand of N1 or its subsequence; and/or (c) encoding a polypeptide having protease activity and having a degree of identity to N1 of at least 96.9 %; (2) a nucleic acid construct (III) comprising (II) operably linked to one or more control sequences that direct the production of the polypeptide in a suitable expression host; (3) a ***recombinant*** expression vector (V1) comprising (III); (4) a ***recombinant*** host cell (IV) comprising (III) or V1; (5) producing (I); (6) a transgenic plant, or plant part, capable of expressing (I); (7) a transgenic, non-human animal, or its products or elements, being capable of expressing (I); (8) an animal feed additive (V) comprising (I), and one or more fat-soluble vitamin, water-soluble vitamin, and/or trace minerals; (9) an animal feed composition having a crude protein content of 50-800 g/kg and comprising (I), or at least one (V); (10) a composition comprising (I), together with at least one other enzyme chosen from phytase (EC 3.1.3.8 or 3.1.3.26), xylanase (EC 3.2.1.8), ***galactanase*** (EC 3.2.1.89), alpha-galactosidase (EC 3.2.1.22), protease (EC 3.4.-.-), phospholipase A1 (EC 3.1.1.32), phospholipase A2 (EC 3.1.1.4), lysophospholipase (EC 3.1.1.5), phospholipase C (EC 3.1.4.3), phospholipase D (EC 3.1.4.4), amylase and/or beta-glucanase (EC 3.2.1.4 or EC 3.2.1.6); and (11) Nocardiopsis prasina DSM 15648.

BIOTECHNOLOGY - Preparation: (I) is produced by cultivating (IV) to produce a supernatant comprising (I) and recovering (I) (claimed).

USE - (I) is useful in animal feed, in the preparation of a composition for use in animal feed, for improving the nutritional value of an animal feed, for increasing digestible and/or soluble protein in animal diets, for increasing the degree of hydrolysis of proteins in animal diets, and/or for the treatment of proteins. (I) is useful in detergents (claimed).

ADVANTAGE - (I) is a low-allergenic ***variant***, capable of invoking reduced immunological response when exposed to animals.

EXAMPLE - Genomic DNA from Nocardiopsis prasina DSM 15648 was isolated, and used as template for PCR amplification using primers having sequences such as 5'-gtcatcgatcgatcgccatggctgcacccggaccgttccccccatgc-3' and 5'-gcggatctattaggccggagacggacgcgcggcaggag-3'. The PCR fragment was isolated on agarose gel (0.7 %). The digested and purified PCR fragment was ligated to the ClaI and BamHI digested plasmid pDG268NeoMCS-PramyQ/PrcryIII/cryIIIAstab/Sav. The ligation mixture was used for transformation into Escherichia coli TOP10F' and several colonies were selected for miniprep. The purified plasmids were checked for insert before transformation into a strain of Bacillus subtilis derived from B. subtilis DN1885 with disrupted apr, npr and pel genes. The transformed host cells were fermented on a rotary shaking table in baffled Erlenmeyer flasks (500 ml) containing PS-1 medium (100 ml) supplemented with chloramphenicol (6 microg/ml), at 37 degreesC for 16 hours and at 26 degreesC for extra 4 days. The culture broth was centrifuged and supernatants were carefully decanted from the precipitates. The combined supernatants were filtered through a Seitz EKS plate to remove the rest of the Bacillus host cells. The EKS filtrate was applied to a bacitracin

silica column, then to G25 Sephadex column and to S sepharose HP column, from which fractions containing protease were eluted. (58 pages)

L5 ANSWER 20 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-03096 BIOTECHDS <<LOGINID::20080204>>

TITLE: New secreted proteolytic polypeptide, useful for e.g.
improving the nutritional value of an animal feed, comprises
non-polar or uncharged polar amino acids within the last four
amino acids of the C-terminus of the polypeptide;
vector-mediated secreted proteolytic protein gene transfer
and expression in host cell for recombinant protein
production and animal feed composition manufacture

AUTHOR: LASSEN S F

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2004111219 23 Dec 2004

APPLICATION INFO: WO 2004-DK431 21 Jun 2004

PRIORITY INFO: DK 2004-335 1 Mar 2004; DK 2003-911 19 Jun 2003

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2005-040099 [04]

AN 2005-03096 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - A secreted polypeptide that has protease activity, is new.

DETAILED DESCRIPTION - A secreted polypeptide comprising at least 3 non-polar or uncharged polar amino acids within the last 4 amino acids of the C-terminus of the polypeptide, and which polypeptide: (a) comprises an amino acid ***sequence*** that is at least 70% identical to the amino acid ***sequence*** of the mature part of the polypeptide comprising any of the 6 sequences having 353-388 amino acids fully defined in the specification; (b) comprises an amino acid ***sequence*** that is at least 70% identical to the amino acid ***sequence*** of the mature part of the polypeptide encoded by the ***polynucleotide*** comprising any of the 8 sequences having 1062-1164 bp fully defined in the specification; (c) comprises a mature part which is a ***variant*** of the mature part of the above polypeptide comprising a substitution, deletion, extension and/or insertion of one or more amino acids; (d) is an allelic ***variant*** of (a), (b) or (c); or (e) is a fragment of any of (a)-(d). INDEPENDENT CLAIMS are also included for the following: (1) an isolated ***polynucleotide*** encoding the above polypeptide; (2) a ***recombinant*** expression vector or ***polynucleotide*** construct comprising the ***polynucleotide*** cited above; (3) a ***recombinant*** host cell comprising the above ***polynucleotide*** or an expression vector or ***polynucleotide*** construct cited above; (4) a transgenic plant or plant part comprising the above ***polynucleotide***, expression vector or ***polynucleotide*** construct; (5) a transgenic non-human animal, or its products or elements, comprising the above ***polynucleotide***, expression vector or ***polynucleotide*** construct; (6) a method for producing the above polypeptide, comprising cultivating the ***recombinant*** host cell, transgenic plant or animal to produce a supernatant comprising the above polypeptide, and optionally recovering the polypeptide; (7) an animal feed additive comprising at least one polypeptide cited above, and at least one fat-soluble vitamin and/or at least one water-soluble vitamin and/or at least one trace material; (8) an animal feed composition having a crude protein content of 50-800 g/kg and comprising at least one polypeptide cited above or at least one feed additive; (9) a composition comprising at least one polypeptide cited above together with at least one enzyme selected from phytase (EC 3.1.3.8 or 3.1.3.26); xylanase (EC 3.2.1.8); ***galactanase*** (EC 3.2.1.89); alpha-galactosidase (EC 3.2.1.22); protease (EC 3.4.--); phospholipase A1 (EC 3.1.1.32); phospholipase A2 (EC 3.1.1.4); lysophospholipase (EC 3.1.1.5); phospholipase C (3.1.4.3); phospholipase D (EC 3.1.4.4); and/or beta-glucanase (EC 3.2.1.4 or EC 3.2.1.6); and (10) a method for using at least one polypeptide cited above for improving the nutritional value of an animal feed, for increasing digestible and/or soluble protein in animal diets, for increasing the degree of hydrolysis of proteins in animal diets, and/or for the treatment of vegetable proteins, comprising including the polypeptide(s) in animal feed and/or in a composition for use in animal feed, or including the polypeptide(s) in a detergent formulation.

BIOTECHNOLOGY - Preferred Polypeptide: The polypeptide is a wildtype polypeptide, an artificial ***variant*** of a wildtype polypeptide, the ***variant*** having one or more amino acid(s) added to the C-terminus as compared to the wildtype, a shuffled polypeptide, or a protein-engineered polypeptide. The one or more added amino acid(s) is/are non-polar or uncharged, such as Gln, Ser, Val, Ala or Pro. The amino acids are selected from Gln-Ser-His-Val-Gln-Ser-Ala-Pro, Gln-Ser-Ala-Pro, Gln-Pro, Thr-Leu, Thr-Thr, Gln-Leu, Thr-Pro, Leu-Pro, Thr-Ile, Ile-Gln, Gln-Pro, Pro-Ile, Leu-Thr, Thr-Gln, Ile-Thr, Gln-Gln and Pro-Gln. The polypeptide which when expressed and before maturation comprises a heterologous pro-region from a different protease, preferably the pro-region is derived from an S2A or S1E protease, more preferably the pro-region is an artificial or shuffled pro-region, and most preferably it is at least 70% identical to the pro-region having any of the 15 amino acid sequences (e.g. 354 or 355 amino acids) mentioned in the specification. The polypeptide which when expressed and before maturation comprises a heterologous secretion signal peptide that is cleaved from the polypeptide when the polypeptide is secreted, preferably the heterologous secretion signal peptide is derived from a heterologous protease. The heterologous secretion signal peptide comprises an amino acid ***sequence*** having a ***sequence*** identity of at least 70% with the amino acid ***sequence*** encoded by polynucleotides 1-81 of a ***sequence*** having 1143 bp fully defined in the specification (SEQ ID NO: 2), or a ***sequence*** having 1164 bp fully defined in the specification (SEQ ID NO: 44). Preferred Host Cell: The host cell is a Bacillus cell.

USE - The composition and methods are useful for improving the nutritional value of an animal feed, for increasing digestible and/or soluble protein in animal diets, for increasing the degree of hydrolysis of proteins in animal diets, and/or for the treatment of vegetable proteins. (115 pages)

L5 ANSWER 21 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-16830 BIOTECHDS <>LOGINID::20080204>>

TITLE: Antimicrobial polypeptide useful for preventing microbial contamination in cooling water systems, laundry rinse water, for preservation of foods, and as a disinfectant e.g. in treatment of acne and infections in the eye or mouth; involving vector-mediated gene transfer and expression in host cell for use in therapy

AUTHOR: SCHINORR K M; WU W

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2004050696 17 Jun 2004

APPLICATION INFO: WO 2003-DK831 4 Dec 2003

PRIORITY INFO: DK 2002-1870 4 Dec 2002; DK 2002-1870 4 Dec 2002

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2004-450720 [42]

AN 2004-16830 BIOTECHDS <>LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - A polypeptide (I) having antimicrobial activity chosen from polypeptide comprising a ***sequence*** which has at least 65% identity with 225 amino acid ***sequence*** fully defined in the specification, or a polypeptide having a ***sequence*** which has at least 65% identity with a polypeptide encoded by the antimicrobial polypeptide encoding part of the nucleotide ***sequence*** present in Rhizomucor pusillus CBS 109471, is new.

DETAILED DESCRIPTION - A polypeptide (I) having antimicrobial activity chosen from a: (a) polypeptide comprising a ***sequence*** which has at least 65% identity with 225 amino acid ***sequence*** (S1) fully defined in the specification; (b) a polypeptide having a ***sequence*** which has at least 65% identity with a polypeptide encoded by the antimicrobial polypeptide encoding part of the nucleotide ***sequence*** present in Rhizomucor pusillus CBS 109471; (c) a polypeptide encoded by a nucleotide ***sequence*** which hybridizes under low stringency condition with a ***polynucleotide*** probe chosen from complementary strand of nucleotides 103-675, 58-675, and 1-675 of a 675 nucleotide ***sequence*** (S2) fully defined in the specification; and (d) a fragment of (a), (b), or (c) that has antimicrobial activity. INDEPENDENT CLAIMS are also included for: (1) a

polynucleotide (II) having ***sequence*** encoding (I); (2) a nucleic acid construct (III) comprising (II) operably linked to one or more control sequences that direct the production of the polypeptide in a suitable host; (3) a ***recombinant*** expression vector (IV) comprising (III); (4) a ***recombinant*** host cell (V) comprising (III); (5) producing (I); (6) ***polynucleotide*** (IIa) having a ***sequence*** which has at least 65% identity with nucleotides 58-675 of (S2); (7) a ***polynucleotide*** (IIb) having a ***sequence*** which has at least 65% identity with antimicrobial polypeptide encoding part of the nucleotide ***sequence*** present in *R. pusillus* CBS 109471; (8) a ***polynucleotide*** (IIc) having ***sequence*** encoding polypeptide having antimicrobial activity, and which hybridize under low stringency condition with a ***polynucleotide*** probe chosen from complementary strand of nucleotides 103-675, 58-675, and 1-675 of (S2); (9) a ***polynucleotide*** (IId) having modified nucleotide ***sequence*** which comprises at least one modification in the polypeptide encoding ***sequence*** of (S2) and where the modified ***sequence*** encodes (S1); (10) an antimicrobial composition (VI) comprising (I); (11) a detergent composition (VII) comprising a surfactant and (I); (12) a transgenic plant (VIII), its part or cell, which has been transformed with (II); (13) an animal feed additive (IX) comprising at least one (I), and at least one fat soluble vitamin and/or water vitamin and/or trace mineral and/or macro mineral; and (14) an animal feed composition (X) having crude protein content of 50-800 g/kg and comprising at least one (I).

BIOTECHNOLOGY - Preparation: Producing (I), involves cultivating a strain, which in its wild-type form is capable of producing (I), and recovering (I), or involves cultivating (V) under conditions conducive for production of (I), and recovering (I) (claimed). Preferred

Polypeptide: (I) has an amino acid ***sequence*** which is 70%, preferably 99% identical to (S1). (I) comprises or consist of (S1). (I) is an artificial ***variant*** which comprises a ***sequence*** that has at least one substitution, deletion and/or insertion of amino acids as compared to (S1). (I) has a ***sequence*** which has at least 70%, preferably 99% identity with a polypeptide encoded by the antimicrobial polypeptide encoding part of the nucleotide

sequence present in *R. pusillus* CBS 109471. (I) comprises or consists of ***sequence*** encoded by the antimicrobial polypeptide encoding part of the nucleotide ***sequence*** present in *R. pusillus* CBS 109471. (I) is an artificial ***variant*** which comprises a ***sequence*** that has at least one substitution, deletion and/or insertion of amino acids as compared to ***sequence*** encoded by the antimicrobial polypeptide encoding part of the nucleotide

sequence present in *R. pusillus* CBS 109471. (I) is encoded by a nucleotide ***sequence*** which hybridizes under medium, preferably high stringency condition with a ***polynucleotide*** probe chosen from complementary strand of nucleotides 103-675, 58-675, and 1-675 of (S2), preferably with a ***polynucleotide*** probe which is complementary strand of nucleotides 58-675 (S2). Preferred Composition: (VI) further comprises an additional biocidal agent. Preferred Feed Additive: (IX) further comprises phytase, xylanase, ***galactanase***, and/or beta-glucanase, and protease.

ACTIVITY - Antimicrobial; Antiseborrheic; Dermatological; Vulnerary.
No biological data given.

MECHANISM OF ACTION - Inhibitor of growth of microbial cells (claimed).

USE - (I) is useful for killing or inhibiting growth of microbial cells. (I) is useful as medicament. (I) is useful as and for preparing antimicrobial veterinarian or human therapeutic or prophylactic agent. (I) is useful in animal feed and for preparation of composition for use in animal feed (claimed). (I) is useful for preventing contamination by bacteria, fungi, yeast or algae in cooling water systems, laundry rinse water, oil systems such as cutting oils, lubricants, oil fields, etc. (I) is useful for preservation of foods, beverages, cosmetics, enzyme formulations, or food ingredients. (I) is useful as disinfectant e.g., in the treatment of acne, infections in the eye or the mouth, skin infections, in antiperspirants or deodorants, in foot bath salts, for cleaning and disinfection of contact lenses, hard surfaces, teeth, wounds, bruises, etc. (I) is useful in cleaning-in-place system for cleaning of process equipment of any kind. (I) is useful for microbial

control of water lines, and for disinfection of water, particularly industrial water. (VI) is useful for production of wood, latex, adhesive, glue, paper, cardboard, textile, leather, plastics, caulking and feed.(50 pages)

L5 ANSWER 22 OF 31 PASCAL COPYRIGHT 2008 INIST-CNRS. ALL RIGHTS RESERVED.
on STN

ACCESSION NUMBER: 2005-0306887 PASCAL <<LOGINID::20080204>>

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TITLE (IN ENGLISH): Effect of the Colorless non-ripening mutation on cell wall biochemistry and gene expression during tomato fruit development and ripening

AUTHOR: ERIKSSON Emma M.; BOVY Arnaud; KEN MANNING; HARRISON Liz; ANDREWS John; DE SILVA Jacquie; TUCKER Gregory A.; SEYMOUR Graham B.

CORPORATE SOURCE: Warwick HRI, Wellesbourne, Warwick CV35 9EF, United Kingdom; Plant Research International, 6700 AA Wageningen, Netherlands; Unilever Research and Development, Colworth, Sharnbrook, Bedford MK44 1LQ, United Kingdom; Division of Nutritional Biochemistry, University of Nottingham, Loughborough, Leics LE12 5RD, United Kingdom

SOURCE: Plant physiology : (Bethesda), (2004), 136(4), 4184-4197, 41 refs.

ISSN: 0032-0889 CODEN: PPHYA5

DOCUMENT TYPE: Journal

BIBLIOGRAPHIC LEVEL: Analytic

COUNTRY: United States

LANGUAGE: English

AVAILABILITY: INIST-3000, 354000138148020310

AN 2005-0306887 PASCAL <<LOGINID::20080204>>

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AB The Colorless non-ripening (Cur) mutation in tomato (Solnum lycopersicum) results in mature fruits with colorless pericarp tissue showing an excessive loss of cell adhesion (A.J. Thompson, M. Tor, C.S. Barry, J. Vrebalov, C. Orfila, M.C. Jarvis, J.J. Giovannoni, D. Grierson, G.B. Seymour [1999] Plant Physiol 120: 383-390). This pleiotropic mutation is an important tool for investigating the biochemical and molecular basis of cell separation during ripening. This study reports on the changes in enzyme activity associated with cell wall disassembly in Cur and the effect of the mutation on the program of ripening-related ***gene*** expression. Real-time PCR and biochemical analysis demonstrated that the expression and activity of a range of cell wall-degrading enzymes was altered in Cnr during both development and ripening. These enzymes included polygalacturonase, pectines-terase (PE), ***galactanase***, and xyloglucan endotransglycosylase. In the case of PE, the protein product of the ripening-related isoform PE2 was not detected in the ***mutant***. In contrast with wild type, Cnr fruits were rich in basic chitinase and peroxidase activity. A microarray and differential screen were used to profile the pattern of ***gene*** expression in wild-type and Cnr fruits. They revealed a picture of the ***gene*** expression in the ***mutant*** that was largely consistent with the real-time PCR and biochemical experiments. Additionally, these experiments demonstrated that the Cnr mutation had a profound effect on many aspects of ripening-related ***gene*** expression. This included a severe reduction in the expression of ripening-related genes in mature fruits and indications of premature expression of some of these genes in immature fruits. The program of ***gene*** expression in Cnr resembles to some degree that found in dehiscence or abscission zones. We speculate that there is a link between events controlling cell separation in tomato, a fleshy fruit, and those involved in the formation of dehiscence zones in dry fruits.

L5 ANSWER 23 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
DUPLICATE 3

ACCESSION NUMBER: 2003-24300 BIOTECHDS <<LOGINID::20080204>>

TITLE: Producing a plant polypeptide in a filamentous fungus (e.g. Aspergillus) host cell comprises introducing into and expressing in the cell a DNA sequence encoding the

polypeptide and that is codon-optimized for expression in the cell;

involving vector-mediated Aspergillus oryzae or Aspergillus niger amylase, Aspergillus niger glucoamylase, Rhizomucor miehei aspartic protease, Humicola insolens cellulase and cutinase and Humicola lanuginosa or Candida antartica lipase gene transfer and expression in fungus cell

AUTHOR: TAIRA R; TSUTSUMI N; TERUI Y; TAKAGI S

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2003070957 28 Aug 2003

APPLICATION INFO: WO 2003-DK108 19 Feb 2003

PRIORITY INFO: DK 2002-871 7 Jun 2002; DK 2002-263 20 Feb 2002

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-671815 [63]

AN 2003-24300 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide of interest obtained from a plant in a filamentous fungus host cell comprising introducing into and expressing in the host cell a DNA ***sequence*** encoding the polypeptide of interest, is new. The DNA ***sequence*** is codon-optimized for expression in the filamentous fungus host cell.

DETAILED DESCRIPTION - Producing a polypeptide of interest obtained from a plant in a filamentous fungus host cell comprising: (a) exchanging at least one codon of the native DNA ***sequence***, the codon encoding an amino acid residue in the polypeptide of interest, with another codon encoding the same amino acid residue of the polypeptide of interest, so that the amino acid ***sequence*** of the polypeptide of interest is unchanged and so that the expression level of the polypeptide of interest is increased in comparison to the expression level in the same host cell and under the same conditions of the polypeptide encoded by the native DNA ***sequence***; (b) introducing into and expressing the DNA ***sequence*** obtained in step (a) in the host cell; (c) culturing the cell obtained in step (b) under conditions conducive to the production of the polypeptide of interest; and (d) isolating the polypeptide of interest. INDEPENDENT CLAIMS are also included for: (1) a DNA ***sequence*** obtained by the novel method; (2) a DNA construct comprising the DNA ***sequence*** of (1); (3) a ***recombinant*** expression vector which carries the DNA construct of (2); and (4) a host cell transformed with the DNA construct of (2) or vector of (3).

BIOTECHNOLOGY - Preferred Method: Producing a polypeptide of interest obtained from a plant in a filamentous fungus host cell alternatively comprises: (a) determining the frequency of the codons in at least one native DNA ***sequence*** encoding a polypeptide obtained from and expressed by the host cell; (b) providing a DNA ***sequence*** encoding the polypeptide of interest, where at least one first codon encoding an amino acid residue in the polypeptide of interest is exchanged with a second codon encoding the same amino acid residue, the second codon having a higher frequency than the first codon as determined in (a); (c) introducing into and expressing the obtained DNA ***sequence*** in the host cell; (d) culturing the obtained host cell under conditions conducive to the production of the polypeptide of interest; and (e) isolating the polypeptide of interest. The method may also alternatively comprise introducing into and expressing in the filamentous fungus host cell a DNA ***sequence*** encoding the polypeptide of interest, the DNA ***sequence*** being codon-optimized for expression in the filamentous fungus host cell. The host cell comprises a promoter ***sequence*** selected from a TPI promoter, a TAKA/TPI promoter, a NA2 promoter, a NA2/TPI promoter, a TEF1 promoter, and their ***mutant*** promoter, truncated promoter or hybrid promoter. The cell comprises a signal ***sequence*** obtained from the genes Aspergillus oryzae TAKA amylase, Aspergillus niger neutral amylase, A. niger glucoamylase, Rhizomucor miehei aspartic proteinase, Humicola insolens cellulase or cutinase, Humicola lanuginosa lipase or Candida antartica lipase B, and their ***mutant***, truncated and hybrid signal ***sequence***. The host cell is a filamentous fungus cell selected from a strain belonging to the genus of Aspergillus, preferably A. oryzae, A. niger, Aspergillus awamori, or the genus of Fusarium, such as a strain of Fusarium oxysporum, Fusarium graminearum,

Fusarium sulphureum, Fusarium cerealis or Fusarium venenatum. The host cell is preferably Aspergillus host cell comprising a TAKA/TPI promoter ***sequence***, a TAKA signal ***sequence*** and a codon-optimized ***sequence*** encoding the polypeptide of interest. The modification of the DNA ***sequence*** results in an increase by at least 1, preferably at least 500 % in the expression level of the polypeptide of interest by the host cell, compared to the expression level of the polypeptide of interest by the host cell comprising the native DNA ***sequence*** encoding the polypeptide of interest. The modified DNA ***sequence*** encoding the amino acid ***sequence*** of the plant polypeptide of interest has had at least one of the first codons, at least 1 % of the first codons, or at least 5, preferably at least 99 % of the first codons encoding an amino acid substituted with a second codon encoding the same amino acid, the second codon having a higher frequency than the first codon. The modification of the DNA ***sequence*** encoding the polypeptide of interest includes substituting an AGA, AGG, CGA or CGG codon encoding Arginine with a CGC or a CGU codon, or substituting a GGG codon encoding Glycine with a GGC, GGA or a GGU codon, or substituting a CAU codon encoding Histidine with a CAC codon, or substituting an AUA codon encoding Isoleucine with an AUC or AUU codon, or substituting a CUA or a UUA codon encoding Leucine with a CUC, CUU, UUG or a CUG codon, or substituting a UUU codon encoding Phenylalanine with a UUC codon, or substituting an AGU or a UCA codon encoding Serine with a UCC, UCG, UCU or an AGC codon, or substituting a GUA codon encoding Valine with a GUC, GUG or a GUU codon. The polypeptide of interest is an industrial enzyme, such as alpha-amylase, alpha-galactosidase, alpha-glucosidase, aminopeptidase, beta-amylase, beta-galactosidase, beta-glucosidase, carbohydrolase, carbonyl hydrolase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, ***galactanase***, glucoamylase, glucose oxidase, hydrolase, invertase, isomerase, laccase, ligase, lipoygenase, lyase, maltogenic alpha-amylase, mannosidase, mutanase, oxidase, oxidoreductase, pectinolytic enzyme, peptidase, peroxidase, phytase, polyphenoloxidase, protease, ribonuclease, transferase, transglutaminase or xylanase. Preferably, the polypeptide of interest is an enzyme having protease and/or peptidase activity, such as an enzyme having protease D3-beta activity. The nucleotide ***sequence*** encodes a polypeptide obtained from soybean. The nucleotide ***sequence*** comprises a DNA ***sequence*** that is at least 70, preferably 100 % identical to a 1131 base pair ***sequence***, given in the specification.

USE - The method is useful in increasing expression and production of plant polypeptides in filamentous fungi host cells. The DNA ***sequence*** is useful in producing plant polypeptides in improved yields. (All claimed)(46 pages)

L5 ANSWER 24 OF 31 USPATFULL on STN
ACCESSION NUMBER: 2003:167772 USPATFULL <<LOGINID::20080204>>
TITLE: Nucleic acid molecules relating to papaya ripening
INVENTOR(S): Pais, Maria Salome Soares, Lisboa, PORTUGAL
Gonsalves, Dennis, Hilo, HI, UNITED STATES
Balde, Aladje, Monte-Abrao (Queluz), PORTUGAL

NUMBER KIND DATE

PATENT INFORMATION: US 2003115633 A1 20030619
US 7084321 B2 20060801

APPLICATION INFO.: US 2002-121393 A1 20020411 (10)

NUMBER DATE

PRIORITY INFORMATION: US 2001-283008P 20010411 (60)

DOCUMENT TYPE: Utility

FILE SEGMENT: APPLICATION

LEGAL REPRESENTATIVE: Michael L. Goldman, Esq., NIXON PEABODY LLP, Clinton Square, P.O. Box 31051, Rochester, NY, 14603-1051

NUMBER OF CLAIMS: 35

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 7 Drawing Page(s)

LINE COUNT: 2484

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to DNA molecules and DNA constructs which promote papaya fruit ripening. The present invention is also directed to methods for promoting or delaying the ripening of papaya plants through transformation of papaya with DNA constructs containing nucleic acids which encode proteins or polypeptides involved in papaya ripening. The invention also relates to expression systems, host cells, and plants containing such DNA constructs.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 25 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-18272 BIOTECHDS <<LOGINID::20080204>>

TITLE: Novel polypeptide having protease activity, useful in animal feed, in the preparation of a composition for use in animal feed, for improving the nutritional value of animal feed and for treating vegetable proteins;
Thermoascus aurantiacus recombinant enzyme production for use in feedstuff

AUTHOR: WU W; HATZACK F; TANG L

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2003048353 12 Jun 2003

APPLICATION INFO: WO 2002-DK824 5 Dec 2002

PRIORITY INFO: DK 2002-5 3 Jan 2002; DK 2001-1821 7 Dec 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-482714 [45]

AN 2003-18272 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - An isolated polypeptide (I) having protease activity, comprising a polypeptide having at least 80% identity to amino acids (-178)-177, (-159)-177 or 1-177 of a ***sequence*** comprising 355 amino acids, or a polypeptide comprising amino acids -23-353, -23-374, -23-397, 1-353, 1-374, 1-397, 177-353, 177-374 or 177-397 of a ***sequence*** comprising 420 amino acids, is new. The sequences are fully defined in specification.

DETAILED DESCRIPTION - An isolated polypeptide (I) comprises: (i) a polypeptide (P1) having at least 80% identity to amino acids (-178)-177, (-159)-177 or 1-177 of a ***sequence*** (S1) comprising 355 amino acids fully defined in the specification, where S1 is derived from Thermoascus aurantiacus (ii) a ***variant*** of the above polypeptide comprising a substitution, deletion and/or insertion of one or more amino acids (iii) a polypeptide (P2) encoded by a nucleic acid ***sequence*** which hybridizes under low stringency conditions with: (a) mature protease encoding part of the plasmid contained in Escherichia coli DSM 14652; (b) nucleotides 25-1089, 1-1089, 1-1344, 25-1344, 559-1344 or 559-1089 of a ***sequence*** (S2) comprising 1344 nucleotides fully defined in the specification; (c) a subsequence of the above nucleotide sequences comprising at least 100 nucleotides; or (d) complements of the above nucleotide sequences; (iv) an allelic ***variant*** of the above polypeptides; (v) a fragment of the above polypeptides that has protease activity or a polypeptide derived from Aspergillus oryzae, comprising amino acids -23-353, -23-374, -23-397, 1-353, 1-374, 1-397, 177-353, 177-374 or 177-397 of a ***sequence*** (S3) comprising 420 amino acids fully defined in the specification, and encoded by nucleotides 2-1129, 2-1195, 2-1267, 71-1129, 71-1195, 71-1267, 599-1129, 599-1195 or 599-1267 of a ***sequence*** (S4) comprising 1267 nucleotides fully defined in the specification. INDEPENDENT CLAIMS are also included for the following: (1) an isolated nucleic acid ***sequence*** (II): (i) comprising a ***sequence*** encoding (I); (ii) encoding a polypeptide comprising protease activity, and hybridizes under low stringency conditions with: (a) a mature protease encoding part of the plasmid contained in E. coli DSM 14652; (b) nucleotides 25-1089, 1-1089, 1-1344, 25-1344, 559-1344 or 559-1089 of S2; (c) a subsequence of the above nucleotide sequences comprising at least 100 nucleotides; or (d) complements of the above nucleotide sequences; and/or (iii) encoding a polypeptide having protease activity and having 70% identity to nucleotides 25-1089, 1-1089, 1-1344, 25-1344, 559-1344 or 559-1089 of S2; (iv) comprising nucleotides 2-1195, 2-1267, 71-1129, 71-1195, 71-1267, 599-1129, 599-1195 or 599-1267 of S4; or (v) produced by hybridizing a

DNA under low stringency conditions with (ii) and isolating the nucleic acid ***sequence*** ; (2) a nucleic acid construct (III) comprising (II) operably linked to one or more control sequences that direct the production of (I) in a suitable expression host; (3) a ***recombinant*** expression vector (IV) comprising (III); (4) a ***recombinant*** host cell (V) comprising (III) or (IV); (5) producing (I); (6) Thermoascus aurantiacus CGMCC No. 0670; (7) an animal feed additive comprising (I), and at least one fat-soluble vitamin, water-soluble vitamin, trace mineral and/or macro mineral, and optionally further comprising phytase, xylanase, ***galactanase*** and/or beta-glucanase; and (8) an animal feed composition having a crude protein content of 50-800 g/kg, and comprising (I).

WIDER DISCLOSURE - Also disclosed are: (1) polypeptides having at least 64% identity to S1; (2) a ***mutant*** of (II); (3) transgenic plant, plant part or plant cell transformed by (II); (4) progeny of the above plant part or cells; (5) transgenic non-human animal and products or elements of the animal comprising (II); and (6) composition comprising (I).

BIOTECHNOLOGY - Preparation: (I) is produced by cultivating (V) to produce a supernatant comprising the polypeptide, and recovering the polypeptide, or by cultivating a strain of Thermoascus, and recovering the polypeptide (claimed). Preferred Polypeptide: (I) is an EC 3.4.24 metalloendopeptidase, and a family M metalloprotease as defined at pp. 989-991 of Handbook of Proteolytic Enzymes by Barrett et al (eds), Academic Press (1998), and/or has an HEFTH motif. The optimum temperature of (I) is 70degreesC and the optimum pH is 6.0. (I) has a molecular weight a 17-22 kDa.

USE - (I) is useful in animal feed, and/or in the preparation of a composition for use in animal feed. (I) is also useful for improving the nutritional value of an animal feed. (I) is also useful for the treatment of vegetable proteins, by adding (I) to at least one vegetable protein or protein source e.g. soybean (claimed).

ADVANTAGE - (I) is stable over a broad range of pH from pH3 to pH10.

EXAMPLE - Thermoascus aurantiacus CGMCC No.0670 was grown at 45degreesC, 165 rpm for 3 days in CGH1 medium. The mycelium was harvested by centrifugation, and total RNA was extracted from 100 mg mycelium using the RNeasy Mini Kit. The degenerate primers (QSALTTA: T025-3 - 5'-CA(A/G)TC(T/C/A/G)GC(T/C/A/G)CT(T/C/A/G)AC(T/C)AC(A/G)GC-3' and T025-12 - 5'-CA(A/G)AG(T/C)GC(T/C/A/G)CT(T/C/A/G)AC(A/G)AC(A/G)GC-3') were designed based on part of the N-terminal amino acid ***sequence***. The 3' rapid amplification cDNA ends (RACE) kit was used to synthesize the cDNA of AP025. About 5 mg total RNA was used as a template and the Adapter Primer was used to synthesize the first strand of cDNA. Then the cDNA of AP025 was PCR-amplified using the above degenerate primers. Gel analysis of the PCR product revealed a specific band corresponding to a fragment of about 800 bp, using primers AP025-3 and AP025-12. The products were recovered from 1% LMP agarose gel, purified by incubation in a 70degreesC bath, followed by using the PCR Preps DNA Purification System. The concentrations of purified products were determined by measuring the absorbance of A260 and A280 in a spectrophotometer. Then these purified fragments were ligated to the pGEM-T vector. 2-4 microl of the ligation products were transformed into 50 microl JM109 high efficiency competent cells by the heat shock method. Transformation cultures were plated onto LB plates with ampicillin/PTG/X-Gal, and these plates were incubated overnight at 37degreesC. ***Recombinant*** clones were identified by color screening on indicator plates and colony PCR screening. The positive clones were selected and plasmid samples were isolated. Finally, the plasmids were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit and the AB1377 sequencer. The sequencing results showed that the PCR band obtained by using primer AP025-3' as well as the primer AP025-12, was the 3'end ***sequence*** of AP025. Based on the 3'-end ***sequence*** of APO25, three specific primers (APO25-5'-1: 5'-AAGGTATATGGCATTGCGAT-3'; APO25-5'-2: 5'-GCAGCCTTGGTAGCCATAC-3' and APO25-5'-3: 5'-TTGATCCTGAGCGTGACAG-3') were designed for cloning the 5'-end of the ***sequence*** : The RACE system was used to synthesize the 5'-end fragment of APO25. 5 microg total RNA and primer 5'-1 was added for synthesis of the first strand. Then the cDNA was purified with DNA purification system and a polyC tail was added to the 3'-end of the cDNA. The primer 5'-2 was used for the second strand synthesis. Using primer APO25-5'-3 in the 5'RACE system, a

specific band was obtained with the size of a 1000 bp fragment. The PCR-products were purified, ligated into pGEM-T-vector, and transformed to JM109 competent cells, and sequenced. Sequencing confirmed that the 5' end fragment of APO25 had been cloned. A primer for full length cloning was designed on the basis of the above 3'- and 5'-end sequences. Primer ÁPO25-CDS-2 (5'-AAGTCTACCCAGTATCCTGT-3') and AUAP were used for amplifying the full length ***gene*** from cDNA of APO25. A specific band with the size of about 1.4 kb was the result of this amplification. A polA tail was added using Taq DNA polymerase and incubation at 72degreesC for 30 minutes. The dA-tailed fragment was recovered, and the purified fragment was ligated into pGEM-T vector, and transformed into the competent cells (JM109). Positive clones were screened, and plasmid was extracted and sequenced. A full-length ***sequence*** was obtained. ***Sequence*** analysis of the cDNA ***clone*** showed a coding region of 1065 nucleotides. The translation product of the coding region was a peptide of 355 amino acids. The deduced amino acid ***sequence*** of this ***gene***, with a signal peptide (from aa 1-19), a propeptide (20-178) and a mature peptide (from aa 179-335). (73 pages)

L5 ANSWER 26 OF 31 BIOTECHDS COPYRIGHT 2008 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2003-17741 BIOTECHDS <<LOGINID::20080204>>

TITLE: Novel Plectasin polypeptide having antimicrobial activity, useful for e.g. killing or inhibiting microbial cell growth, for use as a medicament and as antimicrobial therapeutic or prophylactic agent; recombinant protein production for use in disease therapy and plant engineering

AUTHOR: SCHNORR K M; HANSEN M T; MYGIND P H; SEGURA D R; KRISTENSEN H H

PATENT ASSIGNEE: NOVOZYMES AS

PATENT INFO: WO 2003044049 30 May 2003

APPLICATION INFO: WO 2002-DK781 20 Nov 2002

PRIORITY INFO: DK 2002-1243 23 Aug 2002; DK 2001-1732 20 Nov 2001

DOCUMENT TYPE: Patent

LANGUAGE: English

OTHER SOURCE: WPI: 2003-457589 [43]

AN 2003-17741 BIOTECHDS <<LOGINID::20080204>>

AB DERWENT ABSTRACT:

NOVELTY - A polypeptide (I) having antimicrobial activity, designated as Plectasin, comprising a ***sequence*** having at least 65% identity to amino acids 1-40 of a ***sequence*** (S1) comprising 95 amino acids fully defined in the specification, or to a polypeptide encoded by an antimicrobial polypeptide encoding part of the nucleotide ***sequence*** present in *Pseudoplectania nigrella* CBS 444.97, is new.

DETAILED DESCRIPTION - (I) has antimicrobial activity, and comprises a ***sequence*** having at least 65% identity to amino acids 1-40 of a ***sequence*** (S1) comprising 95 amino acids fully defined in the specification, where the amino acids are in D or L forms, or a polypeptide encoded by the antimicrobial polypeptide encoding part of the nucleotide ***sequence*** present in *Pseudoplectania nigrella* CBS 444.97, a polypeptide which is encoded by a nucleotide ***sequence*** which hybridizes under low stringency conditions with a ***polynucleotide*** probe (PP) selected from the complementary strand of nucleotides 166-285, 70-285 or 1-285 of a ***sequence*** (S2) comprising 288 nucleotides fully defined in the specification, or a fragment of the above polypeptides having antimicrobial activity.

INDEPENDENT CLAIMS are also included for: (1) a ***polynucleotide*** (II) having a nucleotide ***sequence*** encoding (I); (2) a nucleic acid construct (III) comprising (II) operably linked to one or more control sequences that direct the production of the polypeptide in a suitable host; (3) a ***recombinant*** expression vector (IV) comprising (III); (4) a ***recombinant*** host cell (V) comprising (III); (5) producing (I); (6) a ***polynucleotide*** having a nucleotide ***sequence*** which has at least 65% identity to nucleotides 166-285 of S2, a ***polynucleotide*** having a nucleotide ***sequence*** which has at least 65% identity with the antimicrobial polypeptide (Ia) encoding part of the nucleotide ***sequence*** present in *P.nigrella* CBS 444.97, a ***polynucleotide*** having a nucleotide ***sequence*** which encodes a polypeptide having

antimicrobial activity, and which hybridizes under low stringency conditions with a ***polynucleotide*** probe selected from the complementary strand of nucleotides 166-285, 70-285 or 1-285 of S2, or a ***polynucleotide*** having a modified nucleotide ***sequence*** which comprises at least one modification in the mature polypeptide coding ***sequence*** of S2, where the modified nucleotide ***sequence*** encodes a polypeptide comprising amino acids 1-40 of S1; (7) an antimicrobial composition (C) comprising (I); (8) a detergent composition comprising a surfactant and (I); (9) a transgenic plant, plant part or plant cell, which has been transformed with a nucleotide ***sequence*** encoding (I); (10) an animal feed additive comprising (I), and at least one fat soluble vitamin, water soluble vitamin, trace mineral and/or macro mineral; and (11) an animal feed composition having a crude protein content of 50-800 g/kg and comprising (I).

WIDER DISCLOSURE - Wound healing compositions or products such as bandages, medical devices such as catheters, or anti-dandruff hair products such as shampoos, are also disclosed.

BIO TECHNOLOGY - Preparation: (I) is produced by cultivating a strain, which in its wild-type form is capable of producing (I), to produce (I) and recovering (I), or by cultivating (V) under conditions conducive for production of (I) (claimed). Preferred Polypeptide: (I) has at least 70%, preferably 99% identity with amino acids 1-40 of S1 or (Ia). The polypeptide is an artificial ***variant*** comprising a ***sequence*** that has at least one substitution, deletion and/or insertion of an amino acid as compared to amino acids 1-40 of S1 or (Ia). (I) is encoded by a nucleotide ***sequence*** which hybridizes under medium stringency conditions, preferably under high stringency conditions to PP. Preferred Composition: (C) further comprises an additional biocidal agent. The animal feed additive further comprises phytase, xylanase, ***galactanase*** and/or beta-glucanase.

ACTIVITY - Antimicrobial; Fungicide; Antibacterial; Vulnerary; Antiseborrheic; Dermatological; Antiinflammatory. In order to evaluate whether Escherichia coli was growth inhibited in liquid media upon induction of endogenous Plectasin expression, the following experiment was conducted. Briefly, fresh overnight cultures of cells containing either pDRS-18-plectasin, pDR-18-plectasin, pHH or pHHA plasmid were diluted 300-fold into 150 μl of Luria-Bertani (LB) or LB containing 0.1% arabinose in a microtiter plate and incubated at 37 degrees C with vigorous shaking. The growth curve was monitored by measuring OD(450) at regular intervals using an enzyme linked immunosorbent assay (ELISA) reader. Results showed that Plectasin inhibited 41% cell growth when directed to the periplasm however, it did not affect cell growth when expressed in the cytoplasm.

MECHANISM OF ACTION - None given.

USE - (I) is useful for killing or inhibiting growth of microbial cells, for use as a medicament, for use as an antimicrobial veterinarian or human therapeutic or prophylactic agent, and for use in the preparation of a veterinarian or human therapeutic agent for the treatment of a microbial infection or for prophylactic use. (I) is also useful in animal feed, and in the preparation of composition for use in animal feed (claimed). (I) is useful for protection of wood, latex, adhesive, glue, paper, cardboard, textile, leather plastics, caulking, and feed and for preservation of foods, beverages, cosmetics such as lotions, creams, gels, ointments, soaps, shampoos, conditioners, antiperspirants, deodorants, mouth wash, contact lens products, enzyme formulations, or food ingredients. (I) is useful as a disinfectant, e.g. in the treatment of acne, infections in the eye or mouth, skin infections, in foot bath salts, for cleaning and disinfection of contact lenses, hard surfaces, teeth (oral care), wounds and bruises. (I) is useful for cleaning, disinfecting or inhibiting microbial growth on any hard surface. (I) is also useful for cleaning surfaces and cooking utensils in food processing plants and in any areas in which food is prepared or served such as hospitals, nursing homes, restaurants, especially fast food restaurants and delicatessens, as antimicrobial in food products and as a surface antimicrobial in cheeses, fruits and vegetables and food on salad bars. (I) is also useful for microbial control of water lines, and for disinfection of water, in particular for disinfection of industrial water. (I) or (C) is useful for the manufacture of a medicament for controlling or combating microorganisms, such as fungal organisms or bacteria, preferably gram positive bacteria.

ADMINISTRATION - 0.01-200 mg/kg, preferably 0.1-10 mg/kg, of (I) is administered through oral route.

EXAMPLE - The Plectasin encoding ***sequence*** was amplified from a cDNA library by using 1 μl of cDNA (approximately 10 ng of DNA) as template in PCR reaction with primer 178 (tctggatccaccatgcaatttaccca tcctctc) and primer 179 (tctctcgagcttagtaacacttgcacaaacaaagc). Ten pmol of each primer was used in a 100 μl reaction volume. Annealing temperature was 55°C and extension at 72°C for 1 minute. A total of 35 cycles were run. The Expand High Fidelity PCR System was used. Aliquots of the PCR reaction were separated on a 4% agarose gel. Two distinct bands were seen: The most prominent band at a size of approximately 300 bp and a somewhat weaker band at approximately 350 bp. Both fragments were digested with BamHI and XbaI which cut in the overhangs introduced by the PCR primers. The digested fragments were isolated and cloned into pMT2188, an Aspergillus expression plasmid based on the plasmid pCaHj527 constructed as described in example 7 of Danish Patent application PA 2001 00088. The shorter PCR fragment (comprising a ***sequence*** of 303 nucleotides fully defined in the specification) was found to contain the Plectasin encoding ***sequence*** as determined from the signal trapping experiment. Similarly, the ***sequence*** of the longer PCR fragment (comprising 361 nucleotides fully defined in the specification) was determined to contain the Plectasin encoding ***sequence*** and an additional 58 bp insert. It was noted that the 58 bp insert contained the consensus features of the fungal intron, and the amplification of this product was taken as evidence for incomplete intron removal in the mRNA pool and derived cDNA library. The Aspergillus expression plasmid for the shorter PCR product was named pMT2548. pMT2548 was transformed into *A.oryzae* strain BECh2 and into *A.niger* MBin118. Thirty transformants of each strain were re-isolated twice under selective and noninducing conditions on Czapek minimal plates with sucrose and acetamide. To test for the expression of Plectasin, transformants were grown for 6 days at 30°C in tubes with 10 ml YPM (2% peptone, 1% yeast extract, 2% maltose). Supernatants were run on NuPage 10% Bis-Tris SDS gels. Both Aspergillus strains grew well even when induced for the expression of Plectasin. A distinct band of the size expected for Plectasin was seen in most transformants whereas this band was not seen in the untransformed host strains *A.oryzae* (75 pages)

L5 ANSWER 27 OF 31 PASCAL COPYRIGHT 2008 INIST-CNRS. ALL RIGHTS RESERVED.

on STN

ACCESSION NUMBER: 2003-0407195 PASCAL <>LOGINID::20080204>>

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TITLE (IN ENGLISH): Effect of ethylene on mRNA abundance of three .beta.-galactosidase genes in wild type and mutant tomato fruit

AUTHOR: MOCTEZUMA Edgar, SMITH David L.; GROSS Kenneth C.

CORPORATE SOURCE: Produce Quality and Safety Laboratory, USDA-ARS, Bldg. 002, Henry A. Wallace Beltsville Agricultural Research Center, 10300, Baltimore Avenue, Beltsville, MD 20705-2350, United States

SOURCE: Postharvest biology and technology, (2003), 28(2), 207-217, 25 refs.

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BIBLIOGRAPHIC LEVEL: Analytic

COUNTRY: United States

LANGUAGE: English

AVAILABILITY: INIST-27011, 354000118217000010

AN 2003-0407195 PASCAL <>LOGINID::20080204>>

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AB Recent studies indicate that .beta.-galactosidases may play an important role in fruit development and ripening. The temporal expression patterns of a recently discovered family of tomato .beta.-galactosidase (TBG) genes suggest that accumulation of some TBG mRNAs may be regulated by ethylene. Because of the dramatic changes in mRNA abundance patterns that TBG4, TBG5 and TBG6 exhibit at the onset of fruit ripening (when ethylene production begins), we analyzed the effects of ethylene exposure on expression of these genes at 35 days after pollination (Mature Green) in wild type and three tomato ripening-impaired mutants: ripening inhibitor

(rin), non ripening (nor) and Never ripe (Nr). RNA gel blot analysis showed that 48 h of ethylene treatment increased accumulation of TBG4 mRNA up to 20-fold, except in the nor ***mutant***, suggesting that the NOR ***gene*** product is required for ethylene up-regulation of TBG4. Experiments in which fruit were exposed to ethylene for either 2 or 24 h indicated that the observed TBG4 up-regulation is an indirect response to the ethylene treatment, rather than a primary (or direct) response. Ethylene treatment caused the levels of TBG5 mRNA to decrease 63-93% in wild type and ***mutant*** fruit at the Mature Green stage, but had no effect after the onset of ripening. Ethylene dramatically decreased the mRNA abundance of TBG6 in wild type and all mutants at the Mature Green stage. It was shown previously that the TBG4-encoded enzyme has .beta.-galactosidase and exo- ***galactanase*** activity. In order to elucidate its potential cell wall modifying activity, the TBG5-encoded enzyme was expressed in yeast. .beta.-Galactosidase and exo- ***galactanase*** activity of the TBG5-encoded enzyme were confirmed through a quantified release of galactosyl residues from p-nitrophenyl-.beta.-D-galactopyranoside and from tomato fruit cell wall fractions containing .beta.(1 4)-D-galactan, respectively. The TBG6-encoded product was not successfully expressed in yeast. We present a summary of the potential function of the TBGs studied here and their mRNA accumulation in relation to the climacteric production of ethylene in tomato fruit.

L5 ANSWER 28 OF 31 USPATFULL on STN

ACCESSION NUMBER: 2002:310798 USPATFULL <<LOGINID::20080204>>

TITLE: Enzyme with galactanase activity

INVENTOR(S): Kofod, Lene Venke, Uggerl.o slashed.se, DENMARK
 Kauppinen, Markus Sakari, Copenhagen, DENMARK
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 Clausen, Ib Groth, Hiller.o slashed.d, DENMARK
 Mullertz, Anette, Charlottenlund, DENMARK

PATENT ASSIGNEE(S): Novozymes A/S, Bagsvaerd, DENMARK (non-U.S. corporation)

NUMBER KIND DATE

PATENT INFORMATION: US 6485954 B1 20021126

APPLICATION INFO.: US 2000-723548 20001128 (9)

RELATED APPLN. INFO.: Division of Ser. No. US 1998-137855, filed on 21 Aug 1998, now patented, Pat. No. US 6242237, issued on 5 Jun 2001 Continuation of Ser. No. WO 1997-DK92, filed on 28 Feb 1997

NUMBER DATE

PRIORITY INFORMATION: DK 1996-233 19960301
 DK 1996-235 19960301

DOCUMENT TYPE: Utility

FILE SEGMENT: GRANTED

PRIMARY EXAMINER: Prouty, Rebecca E.

ASSISTANT EXAMINER: Rao, Manjunath N.

LEGAL REPRESENTATIVE: Lambiris, Elias

NUMBER OF CLAIMS: 36

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 0 Drawing Figure(s); 0 Drawing Page(s)

LINE COUNT: 1849

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to an enzyme with galactanase activity, a DNA construct encoding the enzyme with galactanase activity, a method of producing the enzyme, an enzyme composition comprising said enzyme with galactanase activity, and the use of said enzyme and enzyme composition for a number of industrial applications.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 29 OF 31 USPATFULL on STN

ACCESSION NUMBER: 2001:226451 USPATFULL <<LOGINID::20080204>>

TITLE: Enzyme with galactanase activity

INVENTOR(S): Kofod, Lene Venke, Uggerl.o slashed.se, Denmark

Kauppinen, Markus Sakari, Copenhagen N, Denmark
Andersen, Lene Nonboe, Aller.o slashed.d, Denmark
Clausen, Ib Groth, Hiller.o slashed.d, Denmark
PATENT ASSIGNEE(S): Novozymes A/S, Bagsvaerd, Denmark (non-U.S.
corporation)

NUMBER KIND DATE

PATENT INFORMATION: US 6329185 B1 20011211
APPLICATION INFO.: US 1998-129033 19980804 (9)
RELATED APPLN. INFO.: Continuation of Ser. No. WO 1997-DK91, filed on 28 Feb
1997

NUMBER DATE

PRIORITY INFORMATION: DK 1996-234 19960301

DOCUMENT TYPE: Utility

FILE SEGMENT: GRANTED

PRIMARY EXAMINER: Prouty, Rebecca E.

ASSISTANT EXAMINER: Rao, Manjunath N.

LEGAL REPRESENTATIVE: Lambiris, Elias J.

NUMBER OF CLAIMS: 19

EXEMPLARY CLAIM: 1

LINE COUNT: 1151

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to an enzyme with galactanase activity, a DNA construct encoding the enzyme, a method of producing the enzyme, an enzyme composition comprising the enzyme, and the use of the enzyme and enzyme composition for a number of industrial applications.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 30 OF 31 USPATFULL on STN

ACCESSION NUMBER: 2001:82562 USPATFULL <<LOGINID::20080204>>

TITLE: Enzyme with galactanase activity

INVENTOR(S): Kofod, Lene Venke, Ugger.o slashed.se, Denmark

Kauppinen, Markus Sakari, Copenhagen N, Denmark

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Clausen, Ib Groth, Hiller.o slashed.d, Denmark

Mullertz, Anette, Charlottenlund, Denmark

PATENT ASSIGNEE(S): Novo Nordisk A/S, Bagsvaerd, Denmark (non-U.S.
corporation)

NUMBER KIND DATE

PATENT INFORMATION: US 6242237 B1 20010605

APPLICATION INFO.: US 1998-137855 19980821 (9)

RELATED APPLN. INFO.: Continuation of Ser. No. WO 1997-DK92, filed on 28 Feb
1997

NUMBER DATE

PRIORITY INFORMATION: DK 1996-233 19960301
DK 1996-235 19960301

DOCUMENT TYPE: Utility

FILE SEGMENT: Granted

PRIMARY EXAMINER: Achutamurthy, Ponnathapu

ASSISTANT EXAMINER: Rao, Manjunath

LEGAL REPRESENTATIVE: Lambiris, Esq., Elias J., Green, Esq., Reza

NUMBER OF CLAIMS: 34

EXEMPLARY CLAIM: 1

LINE COUNT: 1603

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to an enzyme with galactanase activity, a DNA construct encoding the enzyme with galactanase activity, a method of producing the enzyme, an enzyme composition comprising the enzyme with galactanase activity, and the use of the galactanase enzyme and enzyme composition for a number of industrial applications.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L5 ANSWER 31 OF 31 CAPLUS COPYRIGHT 2008 ACS on STN DUPLICATE 4

ACCESSION NUMBER: 1995:695575 CAPLUS <<LOGINID::20080204>>

DOCUMENT NUMBER: 123:193595

TITLE: Tomato exo-(1.fwdarw.4)-beta.-D- ***galactanase***

Isolation, changes during ripening in normal and
mutant tomato fruit, and characterization of a
related cDNA ***clone***

AUTHOR(S): Carey, Annette T.; Holt, Karen; Picard, Sylvie; Wilde,
Rob; Tucker, Gregory A.; Bird, Colin R.; Schuch,
Wolfgang; Seymour, Graham B.

CORPORATE SOURCE: Horticulture Res. International, Wellesbourne,
Warwick, CV35 9EF, UK

SOURCE: Plant Physiology (1995), 108(3), 1099-107

CODEN: PLPHAY; ISSN: 0032-0889

PUBLISHER: Dekker

DOCUMENT TYPE: Journal

LANGUAGE: English

AB An exo-(1.fwdarw.4)-beta.D-galactanase was isolated from ripe tomato
fruit (*Lycopersicon esculentum* Mill. cv Ailsa Craig and cv Better Boy)
using anion-exchange, gel filtration, and cation-exchange chromatog.
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the most
active fraction revealed a predominant protein band at 75 kD and several
minor bands. A 30-amino acid N-terminal sequence from this 75-kD protein
showed a high degree of homol. with other recently identified
.beta.-galactosidase/galactanase proteins from persimmon and apple fruits
(Kang, I.-K., et al.; 1994) and (Ross, G. S., et al.; 1994) and with the
predicted polypeptide sequence encoded by the ethylene-regulated SR12 gene
in carnation (K. G. Raghethama, K. G., et al.; 1991). The enzyme focused
to a single band of .beta.-galactosidase activity on an isoelectrofocusing
gel at pH 9.8. The enzyme was specific for (1.fwdarw.4)-.beta.-D-galactan
substrates with a pH optimum of 4.5. The only reaction product detected
was monomeric galactose, indicating that the enzyme was an
exo-(1.fwdarw.4)-.beta.-D-galactanase. .beta.-Galactanase activity
increased at the on-set of ripening in normal fruit, but no similar
increase was detected in the nonripening mutants nor and rin. A tomato
homolog (pTom.beta.gal 1) was isolated using the SR12 cDNA clone from
carnation as a probe. This clone showed 73% identity at the amino acid
level with .beta.-galactosidase-related sequences from apple and asparagus
and 66% identity with SR12. PTom.beta.gal 1 is a member of a gene family.
Northern anal. demonstrated that pTom.beta.gal 1 expression was
ripening-related in normal fruits, with lower levels apparent in the
nonsoftening mutants.

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L1 QUE (ARABINOGALACTANASE OR GALACTANASE OR (ARABINOGALACTAN (W)

FILE 'CAPLUS, USPATFULL, BIOSIS, SCISEARCH, BIOTECHDS, PASCAL, MEDLINE,
WPIDS' ENTERED AT 11:29:12 ON 04 FEB 2008

L2 1396 S L1

L3 220 S (GENE OR SEQUENCE OR POLYNUCLEOTIDE OR CLONE OR RECOMBINANT)

L4 38 S (MUTANT OR VARIANT) (S) L3

L5 31 DUP REM L4 (7 DUPLICATES REMOVED)

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